THE EFFECT OF TRANSPORT ON LIVE WEIGHT LOSS, MEAT QUALITY AND BLOOD HAEMATOLOGY IN SLAUGHTER OSTRICHES

by

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DECLARATION

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SUMMARY

The production and export of ostrich meat from southern Africa, to especially the European Union, are increasing rapidly due to the healthy nature of ostrich meat. The European Union has very high standards when importing food products, and it is inevitable that more emphasis is being placed on the production of high quality ostrich meat. Another aspect also of concern to consumers, is the welfare of animals prior to slaughter, and this forces producers to look at ways to decrease stress of animals during the *ante-mortem* period. Research regarding the effect of stress during the *ante-mortem* period, and as a result, on meat quality, haematology and weight loss in ostriches, is lacking and thus the purpose of this study was to investigate the effect of various transport distances, travel conditions and different birds on these factors. *Ante-mortem* stress was measured using serum corticosterone levels (ng/ml), heterophil: lymphocyte (H:L) ratio, white blood cell (WBC) count, aspartate aminotransferase (AST) and creatine kinase (CK), as well as the rate and extent of pH decline in the *M. gastrocnemius*. Special emphasis was also placed on the meat quality parameters drip loss, cooking loss, colour and Warner-Bratzler shear force (kg/1.27 cm diameter). Live weight losses, as well as carcass weight and weight of bruises cut off from each bird were also recorded during various stages of the trials.

The effect of transport distance on the meat quality of ostriches was investigated. Ultimate pH_u measurements were taken at 24 hours *post-mortem*. The muscles of the ostriches from the control group (i.e. birds that were not transported prior to slaughter) had a lower mean pH_u (5.77 ± 0.053) than birds that travelled 60 (5.93 ± 0.053) and 600 km (6.11 ± 0.053), respectively. Differences in meat drip loss percentage were also observed between the three treatments. The birds in the control group (0.40 ± 0.07 %) had the lowest meat drip loss percentage compared to the birds that travelled 60 km (treatment C) (1.36 ± 0.07 %) and 600 km (treatment B) (0.97 ± 0.07 %), respectively, to a commcerical ostrich abattoir. Ostriches that were transported for 600 km (8.13 ± 1.16 %) had a greater percentage live weight loss during the *ante-mortem* period than birds that travelled a distance of 60 km (2.4 ± 2.185 %) to the abattoir, although both groups were deprived of feed for the same period. When the haematology of the groups that travelled different distances was compared at various time intervals in the *ante-mortem* period, both groups of birds experienced significant increases in WBC, s-AST and s-CK. An increased H:L ratio from pre-transport to post-transport was only evident in the birds that travelled 600 km (treatment B). However, the birds that travelled 60 km were the only group of birds that had significant elevated serum corticosterone levels during the *ante-mortem* period. The increase in the various blood parameters indicates severe physical stress, which negatively affected meat quality.

Another trial also investigated the effect of various farming systems and transport on meat quality and bruising of ostrich carcasses. Ostriches were raised in three different farming systems, i.e. feedlot -, semi-intensive - and free range conditions. Other factors that could maybe impact on stress susceptibility, such as road conditions, floorspace and floor type were also investigated. A significant difference in meat pH_u was found between ostriches that were raised in a feedlot (5.95 ± 0.018) and semi-intensive (6.04 ± 0.033)

environment. The feedlot birds also had the greatest percentage of carcass weight removed due to bruising. The free range birds were the birds that had the lowest floor density per birds and also had the least amount of bruising on their carcasses. Incidently the other two groups (feedlot and semi intensive) were the birds that travelled on the same type of road (mountain pass) in a truck with rubber flooring whilst the free range birds travelled on a straight road in trucks with metal grid floors.The results indicate that the type of farming system didn't have a significant influence on meat quality of ostriches, but that factors such as road conditions, flooring and bird density did play a significant role in the incidence of bruises and injuries obtained during transport.

OPSOMMING

Die produksie en uitvoer van volstruisvleis vanuit suidelike Afrika, na veral die Europese Unie, is gedurig aan die toeneem as gevolg van die gesonde aard van volstruisvleis. Die Europese Unie het baie hoë standaarde wanneer dit kom by die invoer van voedselprodukte en dit is onvermydelik dat meer klem op die produksie van hoë gehalte volstruisvleis gelê word. 'n Ander aspek wat ook kommer wek by verbruikers is die welstand van diere voor slagting en hierdie aspek noodsaak produsente om te kyk na maniere om stres te beperk tydens die periode voor slagting. Navorsing rakende die effek van stres tydens die *ante-mortem* periode, asook vleiskwaliteit, hematologie en gewigsverlies in volstruise as gevolg van vervoer, ontbreek. Die doel van die studie was dus om die invloed van verskillende vervoerafstande, vervoersomstandighede en tipe produksiesisteme op volstruise se stresrespons te ondersoek. Die omvang van *ante-mortem* stres is bepaal deur die serum-kortikosteroon vlakke (ng/ml), heterofiel: limfosiet (H:L) ratio, witbloedsel (WBS) telling, aspartaat aminotransferase AST en creatien kinase CK, asook die tempo en vlak van pH-daling in die *M. gastrocnemius*, te meet. Spesiale klem is gelê op die vleisgehalte parameters kookverlies, drupverlies, kleur en Warner-Bratzler-skeurwaardes (kg/1.27 cm deursnee). Gewigsverlies is aangeteken op verskillende stadiums tydens die proewe. Karkasgewigte en die hoeveelheid gewig afgesny van elke volstruiskarkas a.g.v. kneusings is ook bepaal.

Die eerste studie het die invloed van vervoerafstand op vleiskwaliteit van slagvolstruise ondersoek. Vleis kwaliteit parameters soos pH, drip verlies, kook verlies, taaiheid en kleur is ondersoek. Die pH_u metings is op 24 uur *post-mortem* geneem. Slagvolstruise in die kontrole groep (d.i. -volstruise wat nie voor slagting vervoer is nie) het 'n laer vleis pH_u (5.77 ± 0.05) gehad as voëls wat onderskeidelik 60 km (5.93 ± 0.05) en 600 km (6.11 ± 0.05) ver vervoer is. Verskille in persentasie dripverlies is gesien tussen die vleis van die voëls wat nie vervoer is nie (0.40 ± 0.07 %) en die voëls wat 60 km (1.36 ± 0.07 %) en 600 km ver (0.97 ± 0.07 %) onderskeidelik vervoer is. Volstruise wat vir 600 km (8.13 ± 1.16 %) vervoer is, het 'n groter persentasie lewende gewig tydens die *ante-mortem* periode as voëls wat 60 km (2.4 ± 2.19 %) ver vervoer is na die abattoir, verloor, al was beide groepe weerhou van voer vir dieselfde tydperk. Beide groepe wat vervoer is (60 en 600 km) het merkbare toenames in witbloedsel (WBS) tellings, s-AST's en s-CK's getoon tydens die ante-mortem periode. Daar is slegs 'n toename in H:L ratio ('n indikator van stres) van voor vervoer tot na vervoer gesien in die voëls wat 600 km vervoer is. Daarteenoor was die voëls wat slegs 60 km vervoer is die enigste voëls wat 'n toename in korticosteroon vlakke getoon het gedurende die *ante-mortem* periode. Die toenames is heel moontlik 'n aanduiding van erge fisiese stres wat 'n negatiewe effek op vleiskwaliteit het.

Die tweede studie het die effek van verskillende produksiesisteme en die stress respons van die verskillende groepe slagvolstuise op vervoer ondersoek. Vleis kwaliteit parameters soos pH, drip verlies, kook verlies en taaiheid is ondersoek. Die hoeveelheid kneusings per volstruis is ook gemeet. Daar was 'n beduidende verskil (P = 0.009) tussen die pH_u van die voerkraal (5.95 ± 0.018) en semi- intensiewe (6.04 ± 0.033)

volstruise. Die voerkraal volstruise se vleis het die grootste drip- en kookverliese gehad in vergelyking met die ander twee groepe (semi-intensiewe en ekstensiewe) terwyl die ekstensiewe volstruise die taaiste vleis gehad het. Die voerkraalvoëls het ook die grootste persentasie karkasgewig verloor a.g.v. kneusings wat afgesny is. Die ekstensiewe voëls het die laagste vloer digtheid per volstruis gehad asook die minste kneusings. Die ander twee groepe (voerkraal en semi intensief) was die groepe wat op dieselfde pad vervoer is (bergpas) in vragmotors wat rubber vloere gehad het terwyl die ekstensiewe voêls op ‘n reguit pad vervoer is in ‘n vragmotor met ‘n metaal oppervlakte. Die resultate van die studie is ‘n aanduiding dat die tipe plaassisteem nie ‘n groot impak op die hoeveelheid akute stres ervaar deur die voëls tydens vervoer gehad het of gevolglik op die vleiskwaliteit van die volstruise nie, maar dat faktore soos pad toestand, tipe vloer en voëldigtheid wel ‘n wesenlike rol speel in die voorkoms van kneusings en beserings opgedoen tydens vervoer.

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LIST OF ABBREVIATIONS

°C	degrees Celsius
a*	red-green colour range
ACTH	adenocorticotrophic hormone
ADP	adenosine diphosphate
ALT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
b*	blue-yellow colour range
C*	chroma
CBG	corticosteroid-binding globulins
CK	creatine kinase
DFD	dark, firm and dry
DM	dry matter
EDTA	ethylene diamine tetraacetic acid
EIA kit	ELISA kit
FAO	Food and Agricultural Organisation
Fisher LSD	Fisher least significant difference
g	gram
GCs	glucocorticoids
GGT	gamma-glutamyltransferase
H_{ab}	hue angle
Hb	haemoglobin
HCT	haematocrit
H:L	heterophil:lymphocyte
HPA	hypothalamic-pituitary-adrenal
Kg	kilogram
KKLK	Klein Karoo Landbou Koöperasie
Km	kilometre
kN	kiloNewton
L*	lightness
LDH	lactate dehydrogenase
LWCC	Livestock Welfare Coordinating Committee
m^2	square meter
MCV	mean cell volume
MCH	mean corpuscular haemoglobin
MJ kg^{-1}	mega joule per kilogram

N	Newton
NEFA	non-esterified fatty acids
ng/ml	nanograms per millilitre
NK	natural killer cells
NOPSA	National Ostrich Processors of South Africa
NS	non significant
$pH_{(1\ hour)}$	pH after 1 hour *post-mortem*
pH_{24}	pH after 24 hours *post-mortem*
$pH_{(30\ min)}$	pH after 30 minutes *post-mortem*
PCV	packed cell volume
pH_u	ultimate pH *post-mortem*
Proc GLM	procsimate generalised linear model
PSE	pale, soft and exudative
RBC	red blood cells
rpm	revolutions per minute
ROS	reactive oxygen species
SAOBC	South African Ostrich Business Chamber
SAOPO	South African Ostrich Producers Organisation
SD	standard deviation
SE	standard error
SST	serum separator tube
TME_N	true metabolisable energy
TP	total protein
vs	versus
WBC	white blood cells
WHC	water-holding capacity

NOTES

This thesis represents a compilation of manuscripts; each chapter is an individual entity and some repetition between chapters, especially in the Materials and Methods section, is therefore unavoidable.

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Chapter 1

INTRODUCTION

Ostrich farming in South Africa started in the 1860s to supply feathers to the feather industry (Smit, 1963). However, over the past few decades, ostrich farming has become more focused on producing meat, with ostrich meat now generating about 60% of the income of ostrich products (Brand, 2010). The increasing demand for ostrich meat is due to the favourable fatty acid profile and low cholesterol content of these animals. Consumers today are more aware of the nutritional characteristics of meat and regard ostrich meat as a healthier alternative that could decrease the effect of coronary heart disease (Cooper & Horbanczuk, 2002). For producers of ostrich meat, ostrich farming also became a viable enterprise in semi-arid and unproductive areas of South Africa (Cooper & Horbanczuk, 2002). This is due to the adaptability and hardy nature of these birds (Stewart, 1994).

Ostrich meat can compete with other livestock species for meat production (Brown & Thompson, 1996). As previously mentioned, ostrich meat has been shown to be much lower in fat content than many other meat-producing livestock species with higher levels of polyunsaturated fatty acids (PUFA) (Horbanczuk *et al*., 1998). This creates the potential for ostrich meat to be marketed under a health banner and according to Lambrechts and Kruger (2006), exports to the European Union have increased up to R1.2 billion. The production of ostrich leather and feathers as fashion items also increases the income generated from slaughter ostriches. As ostrich meat consumption increases (Brown & Thompson, 1996), the drive to increase production and quantity has to be accompanied by measures to improve the quality of ostrich meat. According to Hoffman *et al*. (2007), there is a shortage of literature on factors influencing ostrich meat quality.

The relatively wild behaviour, bi-pedal nature and postural instability of the two-legged ostrich make the transportation of these animals more complex than other livestock species that have four feet and are able to maintain their balance easier (Wotten & Hewitt, 1999). The stressful nature of transport coupled with difficulty in balancing on trucks during transit stresses birds, adversely affecting meat quality and leading to bruising and even mortalities during transport (Reiner *et al*., 1996; Wotten & Hewitt, 1999; Hoffman *et al*., 2010). In order to decrease live weight losses, bruising and mortalities, and increase the quality of ostrich meat, it is becoming vitally important that ostriches are transported in a way that causes minimal *ante-mortem* stress and maximises the meat quality potential of the animal.

According to Sales and Mellet (1996) a high pH_u is a common phenomenon in ostrich muscles and it would appear as if the high pH_u is a characteristic intrinsic to this species (Sales & Mellet, 1996). They classified ostrich meat as moderately to extremely dark, firm and dry (DFD), a characteristic that causes problems for producers and suppliers of ostrich meat. Sales and Oliver-Lyons (1996) described ostrich meat as dark,

whilst Harris *et al*. (1994) reported that ostrich meat was drier in comparison to beef loin steak. Balog and Almeida Paz (2007) proposed that the perceived characteristics of ostrich meat by consumers, such as DFD, are caused by stress during the *ante-mortem* period of the animal. Dark, firm and dry meat also causes favourable conditions for the proliferation of microorganisms that decrease the shelf life of the product, thereby preventing the export of ostrich meat as a fresh product (Balog & Almeida Paz, 2007).

In order to minimise *ante-mortem* stress in ostriches, sufficient research on the conditions that increase stress during this critical period is firstly required. There is currently limited research on the effect of transport stress on ostriches and its effect on meat quality, haematology and other factors such as live weight loss (Mitchell *et al*., 1996; Van Schalkwyk *et al*., 2005; Fasone *et al*., 2005), and few solutions have been found or attempts being made to decrease *ante-mortem* stress. Farmers have also complained about greater weight losses in birds that travelled longer distances to abattoirs compared to birds that travelled shorter distances. However, farmers haven't had sound scientific evidence to base their claims on. It is also important that the full effect of transport stress on meat quality and haematology be investigated to determine if there is a need to improve the current transport practices. Apart from transport distance and production system, other factors such as road conditions, area per bird on the truck, truck flooring and bird handling should also be taken into consideration, as these factors will also potentially influence stress susceptibility, meat quality and bruising of the birds.

This study was therefore conducted to determine the effect of transport distance, transport conditions and farming systems birds were raised in on the birds' stress susceptibility, in order to ultimately determine the effect *ante-mortem* stress had on ostrich meat quality, haematology, live weight loss and bruising. Birds that travelled 60 and 600 km respectively were compared to one other and to birds that didn't travel prior to slaughter. In another trial, birds that were raised in feedlot, semi-intensive and free range farming systems respectively, were compared to determine the effect of the different farming systems on their stress susceptibility during transport. The reason for these comparisons was the fact that the farmers believed that birds that travel longer distances lose more live weight. Increased transit could also lead to a more stressed bird and ultimately to greater changes in haematology and poor quality ostrich meat. The comparisons between the birds that were raised in different farming systems and the effect of transport stress on these individual groups were performed to investigate if feedlot birds, that were seen as more tame and less susceptible to stress, did indeed encounter less stress during transport and produced better quality meat. With all the groups investigated, other external factors such as road conditions, flooring and bird density were also taken into account where applicable.

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Chapter 2

LITERATURE REVIEW

2.1. OSTRICH FARMING IN SOUTH AFRICA

2.1.1. Description

The living ratites are a group of animals that consists of two species of South American rhea, the emu of Australia, six species of New Zealand kiwi, three species of cassowary in New Guinea and North Eastern Australia and four species of ostrich found in Africa (Van Tuinen *et al*., 1998). All these related species have no keel on the sternum and this characteristic prevents these animals from flying (Van Tuinen *et al*., 1998). The wings of the ostrich have low muscle mass. The marketable meat components of the ostrich carcass are mainly concentrated in the posterior limbs (Cooper & Horbanczuk, 2002). The feathers and skin of the ostrich can also be marketed and sold. The ostrich (*Struthio camelus var. domesticus*) in South Africa is commonly known as the South African Black and has been bred since 1863 (Swart *et al*., 1988). Being the largest living bird in the world, it can weigh up to 150 kg with a height of up to 2.75 m (Brown *et al*., 1982).

Ostriches cannot fly and walk on two long, well-developed, legs. The ostrich has two toes, whilst their wings are poorly developed with almost no pectoral muscles. They possess the ability to run long distances at speeds of up to 50 to 70 km/hour (Cramp, 2001). The male ostrich is mainly black with white wing and tail feathers, whilst female birds are mainly grey with white to grey wing and tail feathers (Brown *et al*., 1982).

2.1.2. History

During the 1860s, in the Karoo and Eastern Cape, ostriches were captured and domesticated for the production of their feathers as fashion items (Smit, 1963). The income generated by the production of feathers stimulated the rapid development of ostrich farming, particularly in the Oudtshoorn area, and the development of ostrich farming reached a climax in the early 20th century, just before the onset of World War I. Ostrich feathers constituted South Africa's fourth highest export commodity in 1913 (Smit, 1963). At the start of World War I, in 1913, ostriches numbered approximately 770 000 – a number which then dropped rapidly to 23 000 by 1930 due to the collapse of the market for ostrich feathers (South African Ostrich Business Chamber, 2004).

Even though a cooperative society for ostrich farming was established in 1925 to stabilise market prices, the ostrich industry still suffered. After World War II, the feather industry revived and a new commodity, the

production of biltong from the meat of ostriches, came into being (Smit, 1963). To regulate feather sales, the Klein Karoo Landbou Koöperasie (KKLK) was formed in 1945 (Drenowatz *et al*., 1995). The KKLK took control of the entire ostrich produce in 1959 and also opened a KKLK abattoir in 1963 that mainly produced biltong (Smit, 1963; Drenowatz *et al*., 1995). The leather industry also expanded and a leather tannery was opened in 1969 (Drenowatz *et al*., 1995).

Feathers are now a secondary product of ostrich farming, whilst leather and meat gained greater prominence from the 1980s onwards. The current (2010) income generated from the ostrich products, feathers, meat and leather is 10 %, 60 % and 30 % respectively (Brand, 2010). The deregulation of the ostrich slaughter market in 1993 led to a steady rise in the total amount of ostriches slaughtered in South Africa (Drenowatz *et al*., 1995; Table 2.1). In 1995, South Africa was responsible for 82 % of the global ostrich slaughter market. From 2000 onwards, the contribution of South Africa to the numbers of ostriches slaughtered annually has decreased to 65 %, mainly due to the competition from other countries (South African Ostrich Business Chamber 2004). Table 2.1 gives the average number of birds slaughtered in the last 10 years annually by NOPSA members.

Table 2.1
The number of birds slaughtered and the gross producer value per bird, in South Africa from 2000 to 2010 (National Ostrich Poducers of South Africa, 2011).

Year	Number of birds slaughtered by NOPSA members
2000/2001	158 603
2001/2002	196 283
2002/2003	175 811
2003/2004	169 821
2004/2005	141 081
2005/2006	150 492
2006/2007	132 543
2007/2008	128 710
2008/2009	136 971
2009/2010	149 615

An indication of how fast the ostrich industry has developed in South Africa and also worldwide, is the fact that South Africa slaughtered 152 000 ostriches in 1993 which was 84.5 % of the global production, whilst in 2002 South Africa slaughtered 340 000 ostrich that constituted only 60.7 % of the global production (Ostrich Section 7 Committee Report, 2003). This means that even though South Africa produced more than double the number of slaughter birds in 2003, our percentage contribution to the market declined by more than 20 %

in 11 years. This is mainly due to the rapid rise of ostrich production in Europe, Australia, Namibia and China.

The reason for the increasing demand for ostrich meat throughout the world is mainly due to the low intramuscular fat content and the favourable fatty acid profile of ostrich meat (Sales, 1996; Claassen, 1991). Ostrich meat is usually marketed as individual muscles such as the *M. iliofibularis* (fan fillet), *M. iliofemoralis* (side strip), *M. iliotibialis cranalis*, *M. femorotibialis accessorius*, *M. fibularis longus*, *M. flexor cruris lateralis*, *M. obturatorius medialis*, *M. gastrocnemius* (big drum) and the *M. iliotibialis lateralis* (Mellett, 1996). Ostriches in South Africa are usually slaughtered at an age of 8-14 months and can yield 35 kg of meat on a carcass of 55 kg (African Black). Ostriches are slaughtered between 8 and 14 months to obtain a high quality meat, optimal leather quality and skin size and a single feather harvest (Morris *et al*., 1995).

The ostrich industry is currently organised under an umbrella organisation, the South African Ostrich Business Chamber (SAOBC), with its offices in Oudtshoorn, South Africa. The SAOBC is supported by two established organisations: the National Ostrich Processors of South Africa (NOPSA) and the South African Ostrich Producers Organisation (SAOPO).

2.1.3. Housing and Habitat

The ideal habitat for ostriches is the open, short-grass plains and semi-desert areas of Africa. Ostriches are also known to inhabit the hot deserts of the Western Sahara and Namibia. They tend to avoid dense woods and tall grass areas (Brown *et al*., 1982). In South Africa, the natural habitats for ostriches are coastal fynbos, Karoo shrub land, desert grassland and semi-arid savannas (Dean *et al*., 1994).

The mating season of ostriches in the Klein Karoo is normally from June to January the following year and this is also the laying period where the female ostrich in this area produces eggs (Smith *et al*., 1995). After the breeding season has started, eggs are collected every day, preferably early in the morning or in the late afternoons. The eggs will then undergo the whole process of sterilising and preparation to enter the artificial incubator in the next few days. Almost every commercial ostrich farm in South Africa makes use of artificial incubators to increase hatching numbers (Ostrich Handbook, 2006).

There are three phases of rearing ostriches in South Africa. Immediately post-hatch, rearing to 4-6 months of age and finally the finishing period, frequently in a feedlot (Verwoerd *et al*., 1998). Post-hatch the small birds are maintained in groups of 20-30 inside a building for 2-7 days. In many cases the floor may be heated or insulated with a carpet and the temperature of the room kept at 30 °C (with oil heaters or asbestos heaters) with adequate ventilation (Verwoerd *et al*., 1998).

After hatching, rearing can be conducted in two ways, namely foster rearing and artificial rearing. After artificial hatching the young ostriches are raised by experienced breeders as foster parents and each parent is able to shelter 10-15 young birds on irrigated lucerne pastures. Alternatively, farmers that have yearling hens as foster parents, can move young birds to a shelter at night, with adequate enclosure and heat, and release them again the following morning (De Kock, 1996). Artificial rearing occurs by regulating temperature in a building under cold conditions or at night for small ostriches in groups of 30-50. These buildings are connected to a small paddock of kikuyu grass or lucerne, where these young birds are placed during the day (Verwoerd *et al*., 1998). The birds stay in-house at night for the first two weeks post-hatch. As they become more adept at regulating their body temperature, they will be kept in the paddocks at night, provided with shelter. They will be kept here until 16 weeks of age (Verwoerd *et al*., 1998).

The third phase of rearing is when the ostriches spend around 7-8 months in a feedlot or on lucerne and natural veldt camps, supplemented with a grower ration. In a feedlot there is zero-grazing and the birds are dependent on a grower ration and chopped lucerne, whilst on a lucerne or natural veldt area, birds are able to graze and the grower ration acts only as a supplementary feed (Verwoerd *et al*., 1998).

In South Africa it is estimated that 80 % of ostriches in the Klein Karoo that are destined for slaughtering, are reared, from three months of age, in feedlots while the rest are raised on pastures (Ostrich Handbook, 2006*)*.

2.1.4. Nutrition

The ostrich evolved over centuries to be a selective grazer (Sauer & Sauer, 1966). Milton *et al*. (1994) suggested that ostriches were herbivores, despite the fact that Williams *et al*. (1993) found small amounts of insect parts and small bones in the stomach digesta of ostriches. The bones may possibly be a source of calcium, which is needed for the formation of egg shells. The ostrich has an oral cavity and a tongue that is connected to a long oesophagus. The oesophagus empties into a sac-like proventriculus that connects to a gizzard (Bezuidenhout, 1986). This muscular gizzard contains small stones that help with the grinding of the food to a paste, before the food enters the small intestine. The ostrich has a very long digestive tract. From the gizzard to the rectum, the average length of the gastro-intestinal tract of a mature bird is 22.4 metres (Bezuidenhout, 1986).

Since the ostrich does not have the enzyme cellulose, it is unable to digest plant fibre through enzymes. The passage rate of digesta in ostriches is similar to that of ruminants. In birds weighing 50 kg, Swart *et al*. (1993a) found that the average passage rate was 47.9 hours. This slow passage rate enables ostriches to digest fibre through post-gastric fermentation. Swart (1988) postulated that the products formed by post-gastric fermentation could supply the metabolisable energy (ME) requirements of adult ostriches. Swart *et al*. (1993a) predicted that an ostrich is able to digest and degrade 38 % of the cellulose and 66 % of the hemicelluloses in its diet.

According to Dean *et al.* (1994), very little is known about the natural diet of the ostrich. It is believed that ostriches feed on low-growing, green vegetation such as forbs, green grasses, seeds, berries and succulents in arid, semi-desert to grassland environments during the day (Kok, 1980; Skadhauge *et al*., 1984; Williams *et al*., 1993; Milton *et al*., 1994). The succulents could supply the water needs of the ostrich if the density of the natural vegetation is low (Skadhauge *et al*., 1984). In summer conditions of 30 °C and higher in Oudtshoorn, an adult ostrich on a complete balanced ration in a feedlot, can consume up to 18 litres of water per day (Skadhauge *et al*., 1995). In the Karoo, succulents can constitute up to 20 % of the ostrich diet (Milton *et al*., 1994). In South Africa, ostriches are also reared on lucerne (alfalfa) pastures for slaughtering in an attempt to keep breeding animals on natural rangeland (Smit, 1963; Dean *et al.*, 1994).

For many years maize has been the primary dietary ingredient in monogastric diets, whilst grains such as barley and oats were kept to a minimum due to their high fibre content that could not be digested as effectively in ostriches as in ruminants (Annison, 1993). Cilliers *et al*. (1997) found that ostriches that were given a diet high in fibrous energy sources such as barley were able to digest these energy sources efficiently with little effect on growth rates when compared to birds that received different combinations of feedstuffs. This confirms that ostriches are able to efficiently digest high fibre diets. However Cilliers and Angel (1999) found that ostrich chicks that were younger than 10 weeks didn't digest fibre sufficiently. This means that with an increase in age there is an increase in the ability of ostriches to digest fibrous feedstuffs.

Common ingredients used in diets of ostriches are: maize, soybean and sunflower oilcake, lupines such as full-fat canola and canola oilcake, fishmeal, lucerne hay and salt bush (Cilliers *et al*., 1995). The enhanced digestibility of the hulls of oil cakes makes a significant contribution to the energy availability of these raw materials. This leads to a decreased need for expensive energy sources in ostrich diets and increased use of inexpensive, readily accessible sources of roughage (Cilliers *et al*., 1995). Cilliers *et al*. (1995) also found that sweet lupines had a higher TME_N than soybean and sunflower oilcakes in ostrich diets. Lucerne is the most common source of feed for ostriches due to its high protein and energy content, with the only drawback being that good quality lucerne is not always readily available as ostrich feed in certain areas.

Ostriches can be fed exclusively on pastures or in feedlots where they will receive a complete balanced ration. One of the difficulties presented by pastures is that the growing ostich's diet is not balanced to supply its specific needs, which frequently leads to a decline in growth and poor feed conversion efficiency. Ostriches raised on lucerne pastures and receiving added concentrates compared favourably to ostriches that only received complete diets in feedlots (Cilliers, 1997).

Milton *et al*. (1994) noted that, under extensive conditions, ostriches do not select plant species that contain sodium or calcium oxalate, high fat, phenolic or tannin contents. For many years urea was thought to be toxic to monogastric animals. Angel *et al*. (1999) fed ostriches 0.4, 0.8 and 1.6 % urea respectively for 30

days and only found increases in the blood urea nitrogen content but no effects on feed efficiency or growth rate. Ostriches also prefer not to eat dead plant material or wood (Williams *et al*., 1993).

2.1.5. Transport to abattoirs and lairage

Ostriches are commonly transported as day-old chicks, three-month old chicks and slaughter birds (12-14 months) (Wotten & Hewitt, 1999). Due to trampling and panic during transport, South African producers place workers/stockmen in the truck compartments with the birds in transit to ensure their welfare (Wotten & Hewitt, 1999). When ostriches are transported in South Africa, the ostriches must be handled and transported in accordance to the LWCC (Livestock Welfare Coordinating Committee) code of practice on the handling and transport of livestock (Livestock Welfare Coordinating Committee, 2000).

According to statistics of the National Ostrich Processors of South Africa (NOPSA) in 2004, South Africa had 558 ostrich registered-for-export farms and ten European Union export-approved abattoirs. The production and processing sectors of the ostrich industry employed approximately 20 000 workers in 2004 (NOPSA, 2004). Although the Southern Cape and Karoo, in particular Oudtshoorn, is the largest producer of ostriches, Provinces such as the Free State, Limpopo, Gauteng, Northern Cape, North West and Mpumalanga also have developed intensive ostrich farming. The leading export-approved ostrich abattoirs are located in Oudtshoorn, Mossel Bay, Graaff-Reinet, Swellendam and Malmesbury (NOPSA, 2004). This means that some farmers have to transport their ostriches for long distances to abattoirs. As will be discussed later, transport has detrimental effects on ostrich skins (due to chafings incurred) and causes stress which results in meat of a lower quality. Transportation of ostriches is also believed to decrease live weight mass and muscle yield because of feed deprival, dehydration and loss of intestinal contents. In South Africa, it is proposed that food is withdrawn 10 hours prior to transportation of ostriches to abbatoirs, in order to reduce faecal output on the floor of the vehicle. The faecal output can cause birds to slip and fall and leads to injuries during transport (Mitchell, 1999). Water, on the other hand, should be available to the ostriches at all times prior to transport (Deeming, 1999).

In South Africa, ostriches are transported by specially-built trailers that are divided into compartments, each carrying 8 - 10 ostriches, whilst there are also adapted stock trucks that can carry more ostriches over long distances (Raines, 1995). Sea transport of ostriches is not a common practice, but may be a mode of transport when ostriches are moved from one continent to another. Ostriches will then be carried in containers supplied with water and feed. Pfitzer and Lambrechts (2001) observed that ostriches transported by sea also manifested signs of stress such as stargazing, aggressiveness and homosexual behaviour. The authors also studied the effect of Haloperidol, a neuroleptic used on game to calm them down during translocation, and found that the ostriches were notably calmer after they received the haloperidol during sea transport. Other drugs such as Pexone and Tomanol have been used on ostriches during transport, but with limited success (Wotten & Hewitt, 1999). Payne (1993) transported ostriches by plane on a 6.5 hour journey

with low light levels and under normal temperature and humidity, and observed that the birds were very calm.

2.2. THE EFFECTS OF TRANSPORT STRESS ON HAEMATOLOGICAL AND BLOOD CHEMISTRY VALUES

2.2.1. Defining stress

Stressors during transport can be categorised as either “irritant” or “intermittent” (Crowther *et al*., 2001). Irritant stressors are defined as stressors that occur over long periods of time, continually, such as vibration, noise, novelty, confinement, movement and heat exposure, and these stressors could have a long-lasting effect on the animal. Flashes of light and the noise of passing cars are seen as intermittent stressors and are able to provoke an immediate stress response, but only for a very short duration. Crowther *et al*. (2001) postulated that it was the irritant stressors that were responsible for the greatest effect on stress levels during transport.

Vibration and movement are commonly experienced by ostriches during transport. As the ostrich is bipedal, with a slaughter weight of about 100 kg and a height of about 2.75 metre, the ostrich has a high centre of gravity. With the movement of the vehicle, the ostrich finds it difficult to keep its posture stable and in an upright, standing position. The ostrich then requires and uses a huge amount of energy to keep upright (Foggin, 1992; Wotton & Hewitt, 1999). This increases fatigue and physical exertion in ostriches, which could lead to a condition called ‘capture myopathy’ or exertional rhabdomyolysis (Wotton & Hewitt, 1999). This condition involves the breakdown of muscle tissue and can often lead to brain damage, paralysis and ultimately death. Birds affected with exertional rhabdomyolysis have high rates of mortality after a stressful encounter and birds may still suffer and die up to two weeks after transport. Foggin (1992) found that exertional rhabdomyolysis may be prevented in some cases if the birds were injected with vitamin E or selenium prior to transport, to delay the rate of tissue breakdown.

Heat stress accompanied by dehydration is two further problems encountered during transportation (Crowther *et al*., 2001). In South Africa chill factors during the transportation of ostriches doesn’t affect birds as much due to the moderate climate of South Africa whilst countries such as Poland chill factors will be of greater importance. The welfare issues surrounding the transport of ostriches are mainly related to the thermal stress the birds endure. During transportation, heat is generated by conduction from the bodies of other birds, as well as radiation from the sun.

High temperatures, combined with high humidity, can be threatening to the ostrich that relies on evaporative cooling (Mitchell & Kettlewell, 1998). Except Kwazulu Natal, regional humidity is relative low whilst humidity within trucks has not been documented. According to Crowther *et al*. (2001), ostriches do not consume water

during transit, although it must also be noted that very few trucks have water/drinking facilities on board – it is only with transportation of a long duration, such as encountered on board ships, that drinking water is supplied to the birds. Mitchell (1999) proposed a space allowance of 0.75 m^2 for adult birds being transported and group sizes of up to eight in a compartment on the truck. A closed vehicle with appropriate ventilation, along with the right stocking density, can alleviate heat stress and noise. By insulating the vehicle, noise from the truck or passing cars will be minimised along with visual images. However, very few trucks used in South Africa for the transportation of slaughter birds are this far advanced in their design. Spraying hot birds with water, while in transit, may not be the answer as this may result in the sudden death of certain birds (Wotton & Hewitt, 1999).

The problems of novelty and noise can be alleviated by transporting ostriches in the dark. Crowther *et al*. (2001) and Mitchell (1999) observed that ostriches tend to sit when it becomes dark, and also reported a drop in heart rate. A slower heart rate may well be an indication that the birds become calmer. Crowther *et al*. (2001) also observed lower ambient temperatures and lower skin temperature during transport in the dark, and this may help to alleviate heat stress. It may also relieve the exertional rhabdomyolyis, reported by Foggin (1992) and Wotton and Hewitt (1999), if the birds sit down more during transport in the dark, since this will result in a saving of energy that is usually expended to remain upright during transport. A problem however is that in the case where all the birds do not sit it will lead to trampling of the birds sitting and cause even more bruising.

2.2.2. Blood Haematologic baseline values

Information about the blood haematologic and biochemical values of ostrich blood components is very important for the correct diagnosis and treatment of diseases that commonly affect ostriches. Unfortunately, incomplete information on the blood haematologic and biochemical values of ostriches is found in scientific studies (Van Heerden *et al*., 1985; Levy *et al*., 1989; Verstappen *et al*., 2002). Blood values are also of use to enable a better understanding of the physiology of the ostrich, and the adaptation of these animals to their environment.

Baseline values for blood constituents for all animals are important in order to compare the observed blood parameters in animals under different conditions. Palomeque *et al*. (1991) studied the haematologic values of four Masai ostriches when they were juveniles (5 months) and adults (17 months) (Table 2.2).

Table 2.2

The mean (± s.d.) haematologic values of juvenile and adult ostriches (*Struthio camelus*) (Palomeque *et al*., 1991)**.**

Blood metabolites	Unit	Juvenile	Adult
Hematocrit	%	37.0 ± 2.1	48.0 ± 2.4
Erythrocytes	$\times 10^6$ cells/mm^3	1.91 ± 0.28	2.42 ± 0.37
Haemoglobin	g/dl	13.3 ± 0.39	15.6 ± 0.89
Mean corpuscular volume	µm^3	35.9 ± 3.1	32.5 ± 3.0
Leukocytes	$\times 10^3$ cells/mm^3	19.5 ± 13.7	21.0 ± 8.0

The packed cell volume (PCV) of the blood is more commonly called the hematocrit (HCT) and refers to the percentage of total blood volume that is occupied by red blood cells (Guyton & Hall, 2006). Palomeque *et al*. (1991) reported that the HCT value for adult Masai birds was higher (48 ± 2.4 %) compared to juvenile birds (37 ± 2.1 %). Their findings compared well to Nahid *et al*. (2006) who reported that the HCT baseline values for ostriches in Sudan were 43.6 ± 3.7 %. In another trial, Mushi *et al*. (1999) reported that the HCT baseline value for adult ostriches were 43.25 ± 1.9 % and this also agreed with the two previous studies by Palomeque *et al*. (1991) and Nahid *et al*. (2006). It can now, with some certainty, be postulated that the baseline HCT for ostriches can range between 37 and 48 %.

Red blood cells (RBCs) are also called erythrocytes, and are the most abundant of all blood cells found in blood. The main function of red blood cells is to supply the body tissues with oxygen *via* the circulatory system (Guyton & Hall, 2006). The RBC counts in juvenile and adult birds were 1.91 ± 0.28 × cells/10^6 mm^3 (1.91 ± 0.28 × 10^{12} cells/L) and 2.42 ± 0.37 × 10^6 cells/mm^3 (2.42 ± 0.37 × 10^{12} /L), respectively (Palomeque *et al*., 1991). Minka and Ayo (2008) reported that a RBC of 6.8 ± 0.9 × 10^{12} cells/L, whilst Mushi *et al*. (1999) reported 2.1 ± 0.2. × 10^{12} cells/L for adult ostriches. The values of Mushi *et al*. (1999) and Palomeque *et al*. (1991) are in a similar range and can be accepted as the normal baseline values for RBC. It is uncertain why Minka and Ayo (2008) reported such a high concentration of RBC in their study.

Haemoglobin (Hb) is an iron-containing metalloprotein in RBCs which binds to oxygen and then transports the oxygen in the blood (Guyton & Hall, 2006). Palomeque *et al*. (1991) determined the Hb values of juvenile and adult birds and reported an Hb concentration of 13.3 ± 0.39 g/dl and 15.6 ± 0.89 g/dl respectively. The increase in Hb concentration in adult birds, when compared to junvenile ostriches, can possibly be ascribed to the decreased volume of blood per unit of body weight in adult birds (Nirmalan & Robinson, 1971). According to Palomeque *et al*. (1991), the relatively high values for RBC, HCT and Hb in ostriches may be an indication that the ostrich has a high capacity for oxygen, which enables them to run at high speeds over long periods. Minka and Ayo (2008) reported that the Hb concentrations for adult ostriches were 13.2 ± 2.0 g/dl, which were similar to the results of Palomeque *et al*. (1991). In another trial Mushi *et al*. (1999) reported that the Hb baseline value for adult ostriches was 16.68 ± 0.93 g/dl. This is considerably lower than suggested by the previous two studies mentioned, and the difference could not be explained.

The mean corpuscular volume or mean cell volume (MCV) is a measure of the average red blood cell volume (Guyton & Hall, 2006). According to Palomeque *et al*. (1991), the MCV of juvenile and adult Masai ostriches were 196.9 ± 31.2 and 201.1 ± 25.1 μm^3 respectively whilst Mushi *et al*. (1999) reported a MCV value of adult ostriches of 205.95 ± 15.0 fl (205.1 ± 15.0 μm^3) in a trial conducted in Botswana. These two trials were the only trials in the literature that reported on MCV. It may be suggested that the MCV value for ostriches ranges between 196.9 and 205.95 μm^3.

The mean corpuscular haemoglobin (MCH) is the average mass of haemoglobin per red blood cell in a sample of blood (Guyton & Hall, 2006). The MCH values for juvenile and adult Masai ostriches were 70.5 ± 9.0 pg and 65.3 ± 7.0 pg respectively (Palomeque *et al*., 1991), whilst Mushi *et al*. (1999) reported a slightly higher value for adult ostriches (79.42 ± 12 pg) in Botswana.

Mushi *et al*. (1999) studied the leukocyte counts of juvenile (1-10 month) and adult (11-18 month) ostriches in Botswana to determine if there was any difference in the white blood cell counts (Table 2.3).

Table 2.3
The mean (± s.d.) leukocyte counts for juvenile and adult ostriches (Mushi *et al*., 1999).

Blood metabolites	Unit	Adult	Juvenile	Significance
WBC	$\times 10^9$ cells/L	5.0 ± 1.8	3.8 ± 1.7	NS
Heterophils	%	60 ± 2.1	62 ± 1.2	NS
Lymphocytes	%	32 ± 2.0	29 ± 1.6	NS
H:L ratio		1.875:1	2.138:1	NS
Eosinophils	%	1 ± 0.2	1 ± 0.2	NS
Monocytes	%	1 ± 0.5	1 ± 0.6	NS
Basophils	%	6 ± 1.4	7 ± 1.4	NS

White blood cells (WBC), or leukocytes, are cells of the immune system that protect the body against foreign materials and infectious diseases (Guyton & Hall, 2006). The function of WBC is that these cells are transported to areas of serious infection and inflammation (Guyton & Hall, 2006). The total white blood cell count consists of the following five different types of leukocytes: lymphocytes, heterophils, monocytes, eosinophils and basophils. The white blood cell count of an animal is a good indication of the health status of the animal (Davis *et al*., 2008) The fact that stress decreases the amount of lymphocytes in animals may suggest that it also depresses their disease resistance (Gross & Siegel, 1983).

The white blood cell counts for juvenile and adult Masai ostriches were noted to be 19.5 ± 13.7 $\times 10^9$ cells/L and 21.0 ± 8.0 $\times 10^9$ cells/L respectively (Palomeque *et al*., 1991). Mushi *et al*. (1999) noted a WBC count for adult ostriches of 5.0 ± 1.8 $\times 10^9$ cells/L (Table 3.3), whilst Minka and Ayo (2008) reported even lower WBC

counts (2.0 ± 0.2 × 10^9 cells/L) for adult ostriches. It is unknown why there is so much variation in WBC counts between the three different trials.

The three major types of lymphocytes are the B cells, T cells and natural killer (NK) cells. Natural killer cells are a part of the animal's innate immune system, whilst T and B cells are the major cell components of the adaptive immune response of the animal (Guyton & Hall, 2006). According to Mushi *et al*. (1999), the relative lymphocyte counts of juvenile and adult ostriches were 29 ± 1.6 and 32 ± 2.0 % respectively, whilst Minka and Ayo (2008) reported similar results in adult ostriches (28.4 ± 2.75 %). In a study on South African Black (domesticated) and Red Neck (wild) adult ostriches, no significant difference in lymphocyte percentage between the two groups (25.9 ± 1.5 and 26.6 ± 2.6 % respectively) was reported (Spinu *et al*., 1999).

Another leukocyte class, the heterophils (the avian equivalent of neutrophils found in other vertebrate species) are also present in blood and helps with the defence mechanism of the animal (Guyton & Hall, 2006). In Table 2.3, the relative heterophil counts of juvenile and adult ostriches are 62 ± 1.2 and 60 ± 2.1 % respectively. This corresponds with the findings of Minka and Ayo (2008), who reported that the heterophil count of adult ostriches was 60.1 ± 2.0 %, as well as Spinu *et al*. (1999), who reported average heterophil counts of 63.3 ± 4.6 and 67.2 ± 4.9 % respectively for South African Black (domesticated) and Red Neck (wild) adult ostriches.

Monocytes function primarily as phagocytes to destroy pathogens that can be harmful to the animal (Guyton & Hall, 2006). Mushi *et al*. (1999) reported that the average relative monocyte counts for juvenile and adult ostriches were 1 ± 0.6 and 1 ± 0.5 %, respectively (Table 2.3). Minka and Ayo (2008) found a similar value (2.2 ± 0.7 %).

Eosinophils mainly act against parasitic infections in the blood by their weak phagocytic function (Guyton & Hall, 2006). The relative eosinophil count of juvenile and adult ostriches was the same (1 ± 0.2 %; Mushi *et al*., 1999) whilst Minka and Ayo (2008) reported a value of 0.2 ± 0.1 % for adult ostriches. Although there is a significant variation in the results found by Mushi *et al*. (1999) and Minka and Ayo (2008), this variation in eosinophil count may be normal for adult ostriches. Spinu *et al*. (1999) reported a significant variation in eosinophil percentage for South African Black (domesticated; 2.87 ± 1.61 %) and Red Neck (wild; 0.09 ± 0.11 %,) adult ostriches. This variation reported by Spinu *et al*. (1999) could indicate that domesticated birds are probably exposed to more parasitic infections endemic to housing facilities.

Basophils are primarily responsible for the inflammation response when it initiates the release of the chemical histamine (Guyton & Hall, 2006). According to Mushi *et al*. (1999), the relative basophil counts for adult and juvenile ostriches were 6 ± 1.4 and 7 ± 1.4 % respectively. These counts were much higher than the 0.2 ± 0.3 % reported by Minka and Ayo (2008). This basophil count reported by Mushi *et al*. (1999) is quite high, regardless of the type of species. The unusually high basophil and eosinophil counts reported by Mushi *et al*. (1999) probably suggest that this ostrich population was not a healthy one. On the other hand,

Spinu *et al*. (1999) reported intermediate values for the basophil count of South African Black and Red Neck adult ostriches (3.29 ± 1.93 and 1.82 ± 1.02 % respectively).

The heterophil:lymphocyte (H:L) ratio is a very reliable indicator of stress in birds (Gross & Siegel, 1983). It is thought that the increase in the H:L ratio is a result of the stress imposed on the birds during handling, and that a relative increase in the inflammatory action of heterophils may also stimulate the release of the glucocorticoid hormone, cortisol. Gross and Siegel (1983) also showed that the H:L ratio was a more reliable indicator of stress during transport than the concentration of the glucocorticoid hormone (corticosterone) after transportation, regardless of the length of the journey. According to Leche *et al*. (2009), the glucocorticoid released during an ACTH challenge in Greater Rheas was corticosterone, and not cortisol, a finding supported by Mitchell *et al*. (1996), who transported ostriches and found a 75 % increase in plasma corticosterone ($P < 0.05$) levels after transportation. These findings indicate that the glucocorticoid hormone most abundant in ratites is corticosterone.

According to Maxwell (1993), stressed birds showed an increase in basophils and heterophils and a decrease in lymphocytes. These changes in haematological values of heterophils and lymphocytes during periods of stress, lead to an increase in the H:L ratio of birds (Gross & Siegel, 1983). Mushi *et al*. (1999) reported a 2:1 H:L ratio for adult ostriches when they did not encounter stress. According to Spinu *et al*. (1999), the H:L ratios of South African Black and Red Neck adult ostriches were 2.72 ± 0.84 and 2.78 ± 0.53 respectively. Mitchell *et al*. (1996) reported an even higher H:L ratio for ostriches at baseline level, with a value of 8.5 ± 3.2. This variation points out the sensitivity of this parameter to indicate stress levels - even at baseline some populations may be stressed as a result of handling, noise, etc. encountered during the sampling procedures.

2.2.3. Blood chemistry baseline values

Various studies report on the blood chemistry baseline values of ostriches. Table 2.4 is a summary of blood chemistry baseline values in adult ostriches.

Table 2.4

The range and mean (± s.d.) of selected constituents of ostrich blood of various authors.

Blood Metabolites	Unit	Range of Van Heerden *et al* (1985)	Reports in other literature sources				Other Authors within the same range
			Minimum adult (mean ± s.d.)	Author	Maximum adult (mean ± s.d.)	Author	
Total Protein	g/L	26-50	34.6 ± 6.0	b	49.9 ± 4.6	c	a,b,c,d,e,h,i
Albumin	g/L	14-30.6	16.3	d	30.6 ± 6.7	c	a,b,c,d
Globulin	g/L		20.8	d	20.8	d	
Alb:Glob			0.53:1 ± 0.05	e	0.53:1 ± 0.05	e	
Creatine	µmol/L	6-64	23 ± 10	f	55.556 ± 8.333	b	a,b,c,f
Corticosterone	ng/ml		4.9 ± 2.9	g	4.9 ± 2.9	g	
Glucose	mmol/L	3.6-14.6	9.1 ± 1.8	f	11.9 ± 1.1	e	a,e,f,h
Lactate	mmol/L		6.37 ± 2.11	h	6.37 ± 2.11	h	
Uric acid	µmol/L	303-664.39	375 ± 39	f	664.39 ± 125.50	i	c,f,h,i
Cholesterol	mmol/L	1.7-3.00	1.7 ± 0.5	f	3.00 ± 0.70	i	f,h,i
Triglycerides	mmol/L	0.6-1.59	0.6 ± 0.2	f	1.59 ± 0.41	h	h,f,j
Total Bilirubin	µmol/L	2.46-6.0	2.46 ± 0.68	i	6.0 ± 1.8	f	c,f,i
LDH	IU/L	240-2187	514.9 ± 286.1	i	1101 ± 470	f	a,f,i
AP	IU/L	150-1384	171.5 ± 45.9	i	478.97 ± 251.02	a	a,f,i
AST	IU/L	100-892	117 ± 12	f	236.97 ± 136.35	a	a,f,i
ALT	IU/L	0-12	1.9 ± 1.4	f	20.62 ± 4.36	i	a,f,i
CK	IU/L	394-2500	603 ± 146	f	2667 ± 1041	e	a,e,f,i
GGT	IU/L	0-6	0.25 ± 0.47	e	1.4 ± 0.8	f	a,e,f

[a] Van Heerden et al. (1985)

[b] Nahid *et al*. (2006)

[c] Quintavalla *et al*. (2001)

[d] Cantu-Martinez *et al*. (2010)

[e] Verstappen *et al*. (2002)

[f] Levy *et al*. (1989)

[g] Mitchell *et al*. (1996)

[h] Bovera *et al*. (2007)

[i] Palomeque *et al*. (1991)

[j] Moniello *et al*. (2005)

The total protein (TP) concentration of blood is the total albumin and globulin present in the blood (Guyton & Hall, 2006). The proteins, especially albumin, play a major role in the transport of hormones, electrolytes, enzymes and vitamins in the blood (Guyton & Hall, 2006). TP concentration increases in male ostriches to the age of 24 months, whilst in females, the concentration of TP remains relatively constant (Quintavalla *et al*., 2001). Costa *et al*. (1993) also found that, in emus, TP concentration increased with age. The low values of total protein in young birds often lead to limb deformities and decreased weight gain; this can be rectified

by giving young birds a feed containing sufficient amounts of quality protein (Quintavalla *et al*., 2001). Van Heerden *et al*. (1985) determined that the TP concentration range was between 26-50 g/L for adult ostriches. In studies by Quintavalla *et al.* (2002), Verstappen *et al*. (2002), Nahid *et al*. (2006) and Bovera *et al*. (2007) on adult ostriches, all the authors reported values that fit into the range determined by Van Heerden *et al*. (1985) for total protein concentration. Palomeque *et al*. (1991) found interesting results, regarding the TP concentration for juvenile and adult Masai ostriches. The total protein content reported by Palomeque *et al*. (1991) was higher in juvenile and adult ostriches, and contradicts the observations made by Quintavalla *et al*, (2001), who suggested that the TP concentrations of ostriches increase with age. The results of Palomeque *et al*. (1991) suggested that the TP concentration in the blood decreased with an increase in age of the ostrich. The TP value for adult birds, in the results of Palomeque *et al*. (1999), however, still fit into the range calculated by Van Heerden *et al*. (1985). The higher TP content of juvenile birds, in the results by Palomeque *et al*. (1991), is probably associated with the high metabolic rates of the juvenile birds, in relation to adult birds, due to the higher protein demands of young birds for tissue and feather growth (Palomeque *et al*., 1991). All the above-mentioned studies on the TP concentrations of adult ostriches fit into the range calculated by Van Heerden *et al*. (1985), and we can thus assume, with a relative degree of certainty, that the total protein baseline concentrations of adult birds would not deviate much from the range of Van Heerden *et al*. (1985).

Albumin and globulin are produced in the liver of animals. Van Heerden *et al*. (1985) determined the range of albumin concentration to be between 14 and 24 g/L in the blood of adult ostriches. The mean value for the albumin concentration in adult birds found by Van Heerden *et al*. (1985) was exactly the same as the mean albumin concentration reported by Nahid *et al*. (2006) and Cantu-Martinez *et al*. (2010). However, Quintavalla *et al*. (2000) found a higher value for albumin than the proposed range of values determined by Van Heerden *et al*. (1985), when they recorded an albumin value of 30.6 ± 6.7 g/L in adult ostriches. Only one study reported on the baseline concentration of globulin in adult ostriches (Cantu-Martinez *et al*., 2010) in Mexico, where the baseline concentration of 20.8 g/L for adult ostriches was recorded. Only one other study reported on the albumin:globulin ratio of ostriches. Verstappen *et al*. (2002) found an albumin:globulin ratio of 0.53:1 ± 0.05 in ostriches and this corresponds to the work done by Cantu-Martinez *et al*. (2010), who also found that the globulin concentration was higher than the albumin concentration in the blood of ostriches.

Creatinine is a by-product of the catabolism of creatine phosphate in muscle. In the study conducted by Van Heerden *et al*. (1985), it is reported that the range for creatine was between 6-64 µmol/L. The mean creatinine baseline values reported by Levy *et al*. (1989), Quintavalla *et al*. (2001) and Nahid *et al*. (2006) all fit into the same range for the creatinine baseline levels reported by Van Heerden *et al*. (1985).

Corticosterone is a hydrophobic hormone that circulates in the plasma of ratites, with the majority of the hormone bound to a carrier protein called the corticosterone binding globulin (Rosner, 1990). Corticosterone hormones are released when an animal encounters a stressful situation and a high level of free

corticosterone is maintained in the blood during a prolonged stress response (as cited by Leche *et al*., 2009 from Berg *et al*., 2002). Ratites use their fast-running ability as an anti-predatory strategy and thus require greater amounts of readily available blood glucose. The glucose is made available by the hyperglycaemic effect of corticosterone to aid the animal in a period when it requires sufficient energy for a flight-type stress response (Leche *et al*., 2009). The baseline value for corticosterone concentration reported by Leche *et al.* (2009) was 3.98 ng/ml in Greater Rhea. This value is comparable to other ratites such as the ostrich, where Mitchell *et al*. (1996) reported a baseline concentration of 4.9 ± 2.9 ng/ml. The baseline value for corticosterone, calculated by Mitchell *et al*. (1996) was the only value found in the literature that reported on corticosterone in ostriches.

Glucose is the primary energy source for the body's cells. Van Heerden *et al*. (1985) found that the baseline values for glucose in the blood of adult ostriches ranged between 3.6-14.6 mmol/L. In three other studies (Levy *et al*., 1989; Verstappen *et al*., 2002; Bovera *et al*., 2007), the baseline glucose value of each study fitted into the range for glucose reported by Van Heerden *et al*. (1985).

In animals, lactate is produced from pyruvate, *via* the enzyme lactate dehydrogenase (LDH), during normal metabolism and exercise. The only available study that reported on the lactate level in adult ostriches found that the baseline lactate concentration was 6.37 ± 2.11 mmol/L for 11-month old ostriches (Bovera *et al*., 2007).

Since all birds are uricotelic and produce uric acid as their main nitrogenous end product during metabolism, uric acid is the best indication of renal activity in ostriches (Palomeque *et al*., 1991). Verstappen *et al*. (2002) reported that the minimum uric acid concentration in adult ostriches was 303 µmol/L whilst Palomeque *et al*. (1991) reported the highest mean uric acid concentration in adult Masai ostriches as 664.39 ± 125.50 µmol/L. The mean uric acid concentration reported by Levy *et al*. (1989) and Quintavalla *et al*. (2001) for adult ostriches fit into the range of minimum and maximum values for uric acid. The high variability of the uric acid baseline concentrations in ostriches seems to be a characteristic of this substance when comparing the values of uric acid reported by the above-mentioned authors.

Cholesterol is a steroid metabolite found in cell membranes and transported in the blood plasma of all animals. It is an essential structural component of mammalian cell membranes, where it is needed to establish proper membrane permeability. Cholesterol is also important for the production of fat-soluble vitamins, bile acids and steroid hormones (Guyton & Hall, 2006). The minimum cholesterol value reported for adult ostriches was 1.7 ± 0.5 mmol/L (Levy *et al*., 1989) whilst the maximum cholesterol value reported was 116.2 ± 27.2 mg/dl (3.00 ± 0.70 mmol/L) (Palomeque *et al*., 1991). Bovera *et al*. (2007) reported a value for cholesterol between the minimum and maximum range reported by the above authors. According to Garcia-Rodriguez *et al*. (1987), a wide variation in cholesterol content of different birds may occur due to the circadian rhythms in some species or the effect of the type of diet on plasma levels of cholesterol.

Triglycerides play an important role in metabolism as energy sources and transporters of dietary fat. When the body requires energy, the hormone glucagon initiates the breakdown of the triglycerides by the lipase enzyme, to release free fatty acids. The lowest triglyceride concentration of adult ostriches was reported by Moniello *et al*. (2005) (0.6 ± 0.2 mmol/L) whilst the highest triglyceride concentration was reported by Bovera *et al*. (2007) (1.59 ± 0.41 mmol/L). Levy *et al*. (1989) reported an intermediate mean triglyceride concentration.

Bilirubin is the breakdown product of haem catabolism that resulted after the breakdown of haemoglobin (Guyton & Hall, 2006). The lowest total bilirubin concentration was 0.144 ± 0.04 mg/dl (2.46 ± 0.68 µmol/L) for adult ostriches (Palomeque *et al*., 1991), whilst the highest concentration was 6.0 ± 1.8 µmol/L (Levy *et al*., 1989). The results reported by Quintavalla *et al*. (2001) showed a similar concentration for total bilirubin to that of Palomeque *et al*. (1991).

Lactate dehydrogenase (LDH) is an enzyme that converts pyruvate the final product of glycolysis, to lactate when oxygen is absent or in short supply. Van Heerden *et al*. (1985) found that the baseline values for LDH were between 240-2187 IU/L. The reports of other authors such as Levy *et al*. (1989) and Palomeque *et al*. (1991) for this enzyme all fitted into the range of LDH calculated by Van Heerden *et al*. (1985).

Alkaline phosphatase (AP) is a hydrolase enzyme that removes phosphate groups from many types of molecules, including proteins. Van Heerden *et al*. (1985) reported that the alkaline phosphatase (AP) baseline value is in the range of 150-1384 IU/L for adult ostriches. The AP baseline concentration of adult ostriches reported by Levy *et al*. (1989) and Palomeque *et al*. (1991) fit into this range, although Palomeque *et al*. (1991) found slightly higher values of AP in juvenile ostriches. Moniello *et al*. (2005) found that the concentration of AP is usually higher in younger animals, due to a more rapid bone metabolism in growing animals. This observation was supported by the findings of Costa *et al*. (1993) who found higher concentrations of AP in young emus compared to adult emus.

Aspartate aminotransferase (AST) is an enzyme found in the mitochondria of almost every cell and plays an important role in amino acid metabolism (Bradbury & Berk, 2000). Aspartate aminotransferase (AST) concentration in plasma is raised when there is acute damage to the liver. Aspartate aminotransferase is also present in red blood cells, cardiac muscle, kidney tissue, brain tissue and skeletal muscle, and any damage to these components may also raise the concentration of AST in the plasma. According to Van Heerden *et al*. (1985) the range for AST is between 100-892 IU/L. Levy *et al*. (1989) and Palomeque *et al*. (1991) also reported AST concentrations that fit into this range.

Alanine aminotransferase (ALT) is an enzyme normally found in the liver. Fluctuation of ALT levels in the blood is considered normal over the course of the day. ALT levels can also increase in response to strenuous physical exercise. Van Heerden *et al*. (1985) reported the range of ALT in adult ostriches as being

between 0-12 IU/L. On the other hand, Palomeque *et al*. (1991) found an AST concentration of 20.62 ± 4.36 IU/L for adult Masai ostriches. Levy *et al*. (1989), however, reported a low mean concentration (1.9 ± 1.4 IU/L) for ALT that fitted the range of Van Heerden *et al.* (1985).

Creatine kinase (CK) is an enzyme found in various tissues and cells. CK catalyses the conversion of creatine, and converts adenosine triphosphate (ATP) to adenosine diphosphate (ADP). According to Van Heerden *et al*. (1985), the range for CK lies between 394-2500 IU/L. In the studies of Levy *et al*. (1989) and Palomeque *et al*. (1991) the baseline concentrations of CK fit into this range, whilst only Verstappen *et al*. (2002) reported major differences in CK concentrations (2667 ± 1041 IU/L). Values for CK, as well as the other enzymes discussed, can vary considerably due to the different levels of exhaustion and handling suffered by the birds, before the blood sample was drawn.

Gamma-glutamyltransferase (GGT) is an enzyme present in the cell membranes of many tissues such as the kidneys and liver. Van Heerden *et al*. (1985) reported the range for GGT to be between 0-6 IU/L. Results of Verstappen *et al*. (2002) and Levy *et al*. (1989) fit within this range for GGT.

2.2.4. Intrinsic Factors affecting Haematology and Blood chemistry baseline values

2.2.4.1. Age

Levy *et al*. (1989) determined the influence of age (1 month to 6 years; species unknown) on ostrich blood biochemical values (Table 2.5). There were 65 male and 61 female ostriches in the trial and they all received the same diet.

Table 2.5

Blood biochemical values (mean ± s.d.) for ostriches of different ages (Levy *et al*., 1989).

Analytes	Unit	1-3 months (n= 32)	4-5 months (n= 31)	6-9 months (n= 42)	12-72 months (n= 21)	P-value
Total Protein	g/dl	3.6 ± 0.8	3.6 ± 0.5	3.7 ± 0.7	1.5 ± 0.7	P < 0.001
Glucose	mmol/L	13.4 ± 3.1	12.7 ± 4.4	15.7 ± 3.5	9.1 ± 1.8	P < 0.01
Triglyceride	mmol/L	0.99 ± 0.5	1.2 ± 0.5	1.1 ± 0.5	0.6 ± 0.2	P < 0.001
Cholesterol	mmol/L	2.6 ± 0.1	2.8 ± 0.8	2.8 ± 0.9	1.7 ± 0.5	P < 0.001
Uric acid	µmol/L	561 ± 160	466 ± 86	365 ± 129	375 ± 39	NS
Bilirubin	µmol/L	7.0 ± 1.3	5.2 ± 2.5	4.0 ± 1.5	6.0 ± 1.8	NS
Creatinine	µmol/L	25 ± 5	31 ± 20	31 ± 17	23 ± 10	NS
AP	U/L	531 ± 198	730 ±332	531 ± 198	330 ± 118	NS
ALT	U/L	1.9 ± 1.5	2.5 ± 1.9	1.5 ± 1.9	1.9 ± 1.4	NS
CK	U/L	708 ± 224	725 ± 125	640 ± 205	603 ± 146	P < 0.001
LDH	U/L	1656 ± 479	1881 ± 762	992 ± 410	1101 ± 470	NS
AST	U/L	126 ± 36	146 ± 24	112 ± 27	117 ± 12	NS
GGT	U/L	2.8 ± 0.7	1.1 ± 0.8	1.0 ± 0.7	1.4 ± 0.8	P < 0.01

Levy *et al*. (1989) and Moniello *et al*. (2005) reported similar results in the concentrations of the parameters associated with energy metabolism, such as glucose, triglycerides and cholesterol, although Levy *et al*. (1989) reported significant differences in two of the four groups of each of the three parameters. The glucose, triglyceride and cholesterol values were also similar to that reported elsewhere (Van Heerden *et al*., 1985). The TP values in the studies by Levy *et al*. (1989) and Moniello *et al*. (2005) were also similar to that reported (Palomeque *et al*., 1991; Okotie-Eboh *et al*., 1992), although Levy *et al*. (1989) found significant differences for total protein in two of the four groups analysed. The significant differences recorded by Levy *et al*. (1989) may be due to the extreme differences in the ages of the birds analysed, ranging from 1 month to 6 years. Levy *et al*. (1989) and Moniello *et al*. (2005) also reported similar values for uric acid and creatinine.

The values for the serum enzymes AST, AP, GGT, ALT, and CK showed significant variations due to age, whilst LDH did not show any difference in the trials of Levy *et al*. (1989) and Moniello *et al*. (2005). The results of Levy *et al*. (1989) and Moniello *et al*. (2005) are also contradictory in some of the serum enzymes analysed. This may be due to the fact that the evaluation of enzymes are less standardised than that of other blood constituents, which may account for the variations between laboratories (Moniello *et al*., 2005). The bilirubin values of Levy *et al*. (1989) and Moniello *et al*. (2005) were also in the same range, but Moniello *et al*. (2005) found that bilirubin concentration increased with age, whilst Levy *et al*. (1989) noted that bilirubin concentration decreased with age.

Moniello *et al*. (2005) reported no differences in blood parameter values at two different blood collection sites on the ostrich. He compared the two most common sites for blood collection on ostriches, namely the jugular vein and the brachial vein.

Mushi *et al*. (1999) compared the haematological parameters of 50 juvenile (1-10 months) and 50 adult Masai ostriches (11-18 months) that received the same diet (Table 2.6).

Table 2.6
Haematology parameters (mean ± s.d.) of ostriches in Botswana (Mushi *et al*., 1999).

Haematology parameters	Unit	Adult (mean ± s.d.)	Juvenile (mean ±s.d.)	P-value
		N = 50	N = 50	
RBC	$\times 10^{12}$ cells/L	2.1 ± 0.2	1.8 ± 0.2	NS
Haemoglobin	g/dl	16.68 ± 0.93	10.9 ± 1.2	P < 0.05
PCV	%	43.35 ± 1.9	36 ± 1.2	P < 0.05
MCV	fl	205.95 ± 15.0	200 ± 18.0	NS
MCH	pg	79.42 ± 12	60.56 ± 5.0	P < 0.05
WBC	$\times 10^{9}$ cells/L	5.0 ± 1.8	3.8 ± 1.7	NS
Heterophils	%	60 ± 2.1	62 ± 1.2	NS
Lymphocytes	%	32 ± 2.0	29 ± 1.6	NS
H:L ratio		2 ± 2	2.13 ± 1.5	NS
Eosinophils	%	1 ± 0.2	1 ± 0.2	NS
Monocytes	%	1 ± 0.5	1 ± 0.6	NS
Basophils	%	6 ± 1.4	7 ± 1.4	NS

The PCV, haemoglobin and MCH values in the juvenile ostrich are all significantly lower than that of the adult ostrich. The same results for these haematologic parameters were reported by Palomeque *et al*. (1991). The mean values for total RBC, MCV and total WBC for adult ostriches were all higher than those of the juvenile ostriches, but the difference was non-significant. The age-related increases in haemoglobin in birds were demonstrated by Nirmalan and Robinson (1971). According to Nirmalan and Robinson (1971), the increases in the concentration of haemoglobin could be due to a change in the blood volume/unit body weight.

2.2.4.2. Diet

The differences in the blood biochemical values of two groups of ostriches (*Struthio camelus* var. *domesticus*) fed different diets are depicted in Table 2.7 (Bovera *et al*., 2007). The birds were 11 months old and had an average weight of 91.4 ± 4.31 kg. There were 40 ostriches in the trial with 20 males and 20 females. Each diet had the same percentage of concentrate (60 % DM), with the only difference in the diets being the type of forage (40 % DM) the groups received. Diet A consisted out of 60 % concentrate and 40 %

corn silage, whilst diet B consisted out of 60 % concentrate and 40 % dehydrated alfalfa. Diet B, however, had a higher percentage of protein (17.3 % DM) compared to diet A (14.4 % DM), which may affect the results.

Table 2.7

The metabolic profile (mean ± s.e.) of ostriches that received different diets (Bovera *et al*., 2007).

Blood metabolite	Unit	Diet A	Diet B	P-value
Glucose	mmol/L	9.54 ± 1.38	9.91 ± 1.65	NS
Cholesterol	mmol/L	1.99 ± 0.61	2.05 ± 0.36	NS
Triglycerides	mmol/L	1.59 ± 0.41	1.53 ± 0.39	NS
Lactate	mmol/L	6.37 ± 2.11	6.63 ± 1.62	NS
Total Proteins	g/L	40.85 ± 7.48	38.35 ± 4.20	NS
Uric acid	mmol/L	222.5 ± 83.5	378.5 ± 75.8	P < 0.01
Creatinine	mmol/L	27.35 ± 7.23	32.6 ± 6.24	P < 0.05
Total Bilirubin	mmol/L	8.50 ± 1.91	10.65 ± 3.8	P < 0.05
AST	U/L	344.9 ± 54.5	461.4 ± 133.8	P < 0.01
ALT	U/L	12.2 ± 2.14	12.8 ± 2.46	NS
Alkaline Phosphatase	U/L	71.9 ± 19.7	72.6 ± 16.1	NS
GGT	U/L	8.50 ± 1.91	11.3 ± 4.27	P < 0.05

The energy metabolism parameters, triglycerides, cholesterol and glucose did not differ significantly between the two diets and were comparable with previous studies of Van Heerden *et al*. (1985), Levy *et al*. (1989), and Okotie-Eboh *et al*. (1992).

There was no significant difference in total protein content between the two groups, which is in agreement with previous studies (Van Heerden *et al*., 1985; Levy *et al*., 1989; Palomeque *et al*., 1991; Okotie-Eboh *et al*., 1992; Moniello *et al*., 2005). The values for uric acid and creatinine were, however, significantly higher for birds fed diet B.

The values for lactate, ALT and alkaline phosphatase were not significantly different in the two groups and were similar to results published (Okotie-Eboh *et al*., 1992; Moniello *et al*., 2005). The AST and GGT values were significantly higher in the group fed the B diet, but in each case the values are similar to the results reported by Moniello *et al*. (2005). However, it seems that the values for GGT differ greatly in literature (Van Heerden *et al*., 1985; Levy *et al*., 1989; Okotie-Eboh *et al*., 1992). The values for bilirubin were also significantly higher in birds fed diet B.

According to Costa *et al*. (2008), blood TP content does not change with changes in protein content of the feed, but uric acid serum levels increase due to higher protein levels in the diet, as can be seen in the study by Bovera *et al*. (2007). The reason for the higher levels of creatinine is uncertain, but creatinine is

considered an index of muscle metabolism and higher values of creatinine may indicate an accelerated muscle tissue turnover (Finco, 1989). Bovera *et al*. (2007) suggested that the higher levels of AST due to diet B could be related to the increase in creatine and muscle metabolism. GGT is positively related to cell damage of the liver tissue. The higher protein diet B could well lead to liver damage, due to the greater involvement of the liver in protein metabolism in the birds receiving diet B.

No literature could be sourced that reported on the effect of different diets on the haematological and immunological variables in ostrich blood.

2.2.4.3. Species

There are a few studies that researched the haematology and blood chemistry values of ostriches at various ages, but in most of the literature the species of the ostrich were not noted. This makes it difficult to compare haematologic and blood chemistry values of ostriches from different regions such as the South African Black, Blue and Red Neck and East African Masai ostriches. However, comparisons were made between the ostrich, Lesser Rhea and emu on the basis of haematology and blood chemistry values. All these animals belong to the species of ratites (Van Tuinen *et al*., 1998).

Table 2.8 and Table 2.9 contain the haematologic values of Lesser Rhea and emus respectively. Chang Reissig *et al*. (2002) evaluated blood from juvenile (8 - 24 months) and adult (over 24 months of age) Lesser Rhea (Table 2.8).

Table 2.8
The haematology values of Lesser Rhea juveniles and adults (Chang Reissig *et al*., 2002).

Haematological Parameters	Unit	Juveniles (n = 24)	Adults (n = 36)	P - value
RBC	$\times 10^{12}$/L	2.3 ± 0.2	2.2 ± 0.2	$p < 0.05$
WBC	$\times 10^{9}$/L	13.5 ± 5.1	14.1 ± 3.6	ns
PCV	%	0.48 ± 0.04	0.48 ± 0.04	$p < 0.05$
Hb	g/L	178 ± 19	178 ± 23	$p < 0.05$
MCV	fL	218 ± 18	219 ± 19	$p < 0.05$

In Table 2.9, the haematological parameters from male and female emu birds at 18 month of age, when sexual maturity occurred, are reported (Patodkar *et al*., 2008).

Table 2.9

Mean haematological parameters (mean ± s.e.) of adult male and female emus (Patodkar *et al*., 2008).

Haematological Parameters	Unit	Male (n=7)	Female (n=7)	P-value
Total Erythrocyte count	10^6 cells/mm^3	1.79 ± 0.08	1.36 ± 0.05	P < 0.01
Total leukocyte count	10^3 cells/mm^3	17.21 ± 1.19	14.31 ± 0.84	NS
Haemoglobin	g/dl	11.97 ± 0.17	11.8 ± 0.59	NS
PCV	%	37 ± 2.25	36.71 ± 1.86	NS
Heterophil	%	63 ± 2.16	63.43 ± 2.13	NS
Lymphocyte	%	31.57 ± 2.25	32.71 ± 1.8	NS
Eosinophil	%	2.71 ± 0.36	2.29 ± 0.36	NS
Basophil	%	1 ± 0.31	0.71 ± 0.29	NS
Monocyte	%	1.71 ± 0.36	0.86 ± 0.34	NS

Both ratite species (mentioned above) had similar RBC concentrations than the adult ostriches (2.1 ± 0.2 × 10^{12} cells/L) in the study by Mushi *et al*. (1999).

The WBC concentrations of adult Lesser Rheas were determined to be between 7.5-25.7 × 10^9/L (Chang Reissig *et al*., 2002) whilst Patodkar *et al*. (2008) reported a value of 17.21 ± 1.19 10^3 cells/mm^3 (17.21 ± 1.19 × 10^9 cells/L) for emus. All these values were higher than the value reported for adult ostriches (5.0 ± 1.8 × 10^9/L) by Mushi *et al*. (1999).

Chang Reissig *et al*. (2002) reported that the packed cell volume (PCV) value for adult Lesser Rheas was between 43-55 % and that the mean value was 48 ± 4 %. Patodkar *et al*. (2008) reported a significantly lower PCV value for emus (37 ± 2.25 %). In adult ostriches Mushi *et al*. (1999) reported a PCV value of 43.25 ± 1.9 %.

The haemoglobin range for Lesser Rheas was calculated by Chang Reissig *et al*. (2002) to be within the range of 124-224 g/L (12.4-22.4 g/dl) and the mean value to be 178 ± 23 g/L (17.8 ± 2.3 g/dl). Patodkar *et al*. (2008) reported that the haemoglobin concentration for emus was 11.97 ± 0.17 g/dl. The haemoglobin concentration of both the emu and Lesser Rhea corresponds to the value of 16.68 ± 0.93 g/dl noted for the adult ostrich (Mushi *et al*., 1999).

According to Chang Reissig *et al*. (2002), the mean corpuscular volume (MCV) of Lesser Rheas was in the range of 176-287 fL with a mean value of 219 ± 19 fL. On ostriches, Mushi *et al*. (1999) reported a MCV value of 205.95 ± 15.0 fL that corresponds to the MCV values of Lesser Rheas.

The mean value of heterophils in the study on cassowaries was significantly higher than the values reported by Mushi *et al*. (1999) on ostriches and Patodkar *et al*. (2008) on emus. Mushi *et al*. (1999) and Patodkar *et al*. (2008) reported values of 60 ± 2.1 % and 63 ± 2.16 % respectively.

In ostriches and emus, Mushi *et al*. (1999) and Patodkar *et al*. (2008) reported values of 32 ± 2.0 % and 31.57 ± 2.25 % respectively for lymphocytes. Patodkar *et al*. (2008) and Mushi *et al*. (1999) reported monocyte values of 1.71 ± 0.36 % and 1 ± 0.5 % respectively for emus and ostriches.

The basophil percentage in emus in the study by Patodkar *et al*. (2008) was 1 ± 0.31 %, which was lower than the 6 ± 1.4 % reported on adult ostriches (Mushi *et al*., 1999). The relative eosinophil count for emus was 2.71 ± 0.36 % according to Patodkar *et al*. (2008), whilst the eosinophil count for ostriches was 1 ± 0.02 % (Mushi *et al*., 1999). The heterophil:lymphocyte (H:L) ratio of emus and ostriches were both 2:1 (Patodkar *et al.*, 2008; Mushi *et al.*, 1999).

Okotie-Eboh *et al*. (1992) investigated whether there were any difference between the serum blood biochemical values for emus and ostriches. They analysed 33 emus between the ages of 1 and 48 months and 54 ostriches between the age of 1 and 60 months. Table 2.10 is a summary of the results obtained in their study.

Table 2.10
The blood biochemistry values of emus and ostriches (Okotie-Eboh *et al*., 1992).

		Emus Total (n = 33)		Ostriches (n = 54)		Significant difference in
Analytes	Unit	Mean ± SD	Range	Mean ± SD	Range	species
Glucose	mg/dl	158 ± 22	111 - 218	245 ± 50	171-420	P < 0.05
Uric Acid	mg/dl	4.7 ± 2.0	2.2 - 11.0	9.1 ± 4.0	4.3-26.6	P < 0.05
Creatinine	mg/dl	0.2 ±0.1	0.1-0.4	0.4 ± 0.1	0.2-0.8	P < 0.05
Triglyceride	mg/dl	325 ± 591	54-3137	130 ± 119	48-828	P < 0.05
Cholesterol	mg/dl	104 ± 31	64-196	100 ± 38	36-202	NS
Total Protein	g/dl	4.2 ± 0.5	3.5-6.3	3.8 ± 0.7	2.5-6.1	P < 0.05
Albumin	g/dl	2.5 ± 0.3	1.6-3.2	1.8 ± 0.3	1.2-2.9	P < 0.05
ALT	IU/L	15.4 ± 4.3	7.1-25.9	20 ± 14	3-75	P < 0.05
AP	IU/L	8 ± 44	22-275	150 ± 80	22-363	P < 0.05
AST	IU/L	104 ± 24	78-182	280 ± 84	187-714	P < 0.05
CK	IU/L	264 ± 170	70-818	2470 ± 2059	1002 - 10413	P < 0.05
GGT	IU/L	4.4 ± 3.4	0.2 - 13.7	2.1 ± 2.1	0.1-11.4	P < 0.05
LDH	IU/L	240 ± 91	124 - 469	734 ± 255	345-1416	P < 0.05

Although the ranges for total protein in emus and ostriches overlap, there is a significant difference between the TP values for emus and ostriches.

There were significant differences in all the other blood biochemicals of the emus and ostriches in the study by Okotie-Eboh *et al*. (1992), except in the values of cholesterol. The enzymes ALT, AP, AST, LDH and CK were all significantly higher in the ostrich, which could maybe indicate that ostriches suffered more physical exertion during capture than emus.

2.2.4.4. Sub-species

The differences in haematological counts of the *S. c. domesticus* (South African Black) and the *S. c. camelus* (Red Neck) sub-species are depicted in Table 2.11 (Spinu *et al*., 1999). Twenty blood samples from each of the six ostriches (one sample every second day) were collected. The *S. c. domesticus* (South African Black) originated from a cross between the *S. c. australis* (Blue Neck) and the *S. c. camelus* (Red Neck) as previously described. The ostriches studied were all males of approximately 5 years of age.

Table 2.11
White blood cell differentiation counts of *S. c. domesticus* (South African Black) and the *S. c. camelus* (Red Neck) sub-species (Means ± s.d.) (Spinu *et al*., 1999).

Blood metabolite	Unit	South African Black (n = 3)	Red Neck (n = 3)	P-value
Lymphocytes	%	24.5 ± 2.2	25.3 ± 3.4	NS
Heterophils	%	63.3 ± 4.6	67.2 ± 4.9	NS
Eosinophils	%	2.87 ± 1.61	0.09 ± 0.11	P < 0.05
Basophils	%	3.29 ± 1.93	1.82 ± 1.02	P < 0.05
H:L ratio		2.72 ± 0.84	2.78 ± 0.16	NS

*P (Mann-Whitney U-test)

The total white blood cell count was similar in both groups and the counts also corresponded to other ostrich subspecies (Levy *et al*., 1989; Palmeque *et al*., 1991). As is depicted in Table 2.11, the basophil and eosinophil values varied significantly in the two groups studied. The South African Black birds had higher values for both basophils and eosinophils. Eosinophils play a defensive role against parasites such as protozoa and worms, whilst basophils act as a mediator in the early inflammatory response (Maxwell, 1993).

2.2.4.5. Gender

Levy *et al*. (1989) conducted research on the effect of gender on the blood biochemical values of ostriches (*Struthio camelus*). The ostriches (subspecies unknown) used in the trial consisted of 65 males and 61 females between the ages of 1 and 72 months (Table 2.12).

Table 2.12

Blood biochemistry values for male and female ostriches (Levy *et al*., 1989).

Analytes	Unit	Male (n = 65)	Female (n = 61)	Significance
Total Protein	g/dl	3.8 ± 0.6	3.5 ± 0.8	NS
Glucose	mmol/L	14.1 ± 4.2	11.9 ± 4.4	NS
Triglyceride	mmol/L	0.99 ± 0.5	1.0 ± 0.5	NS
Cholesterol	mmol/L	2.6 ± 0.9	2.4 ± 0.8	NS
Uric acid	µmol/L	476 ± 167	499 ± 166	NS
Bilirubin	µmol/L	5.9 ± 2.4	5.8 ± 1.5	NS
Creatinine	µmol/L	36 ± 34	42 ± 42	NS
Alkaline phosphatase	IU/L	626 ± 285	531 ± 248	NS
Alanine transaminase	IU/L	2.0 ± 1.9	2.1 ± 1.5	NS
Creatine kinase	IU/L	682 ± 205	694 ± 214	NS
Lactate dehydrogenase	IU/L	1575 ± 777	1556 ± 550	NS
Aspartate transaminase	IU/L	125 ± 33	124 ± 31	NS
y- glutamyltransferase	IU/L	1.6 ± 4.0	1.5 ± 0.9	NS

Levy *et al*. (1989) reported that gender had little influence on the blood biochemical values of ostriches (Table 2.12). This finding was supported by Van Heerden *et al*. (1985) who also reported no difference in blood biochemical values of ostriches of different sexes. No literature could be sourced on the effect of sex on the haematological and immunological variables in ostrich blood.

2.2.5. Endocrine response of ostriches to stress

In birds, the response to an uncontrollable stressor, such as transport, depends on the integrative working of the endocrine and nervous system. When the neurogenic system is activated, its primary goal is to combat the stressor, rather than to accommodate it (Siegel, 1980). The neuropeptides, epinephrine (noradrenalin) and norepinephrine are the main activators of this response. The activation of the nervous system causes sudden changes in the body, such as increases in muscle tone, blood pressure and body temperature (Mack *et al*., 1994)

When an animal is exposed to a stressor, an integrated neuroendocrine response is also triggered (Ellis *et al*., 2006). This involves the activation of the hypothalamic- pituitary-adrenal (HPA) system. Even though the activation of the HPA axis is considered to be an adaptive response to help the animal cope with a stressful situation, the continuous stimulation of the axis can have a negative effect on the performance and welfare of animals (Jones, 1996). The HPA system is activated by neural or blood-borne stimuli that increase the production of the corticotrophin-releasing factor. The corticotrophin-releasing factor then stimulates the anterior pituitary gland to increase the production of ACTH (adrenocorticotrophic hormone). The ACTH then

proliferates in the blood and stimulates the adrenal cortical tissue to produce corticosteroid hormones such as corticosterone (Siegel, 1980). Corticosteroids have various effects and these effects are mediated by blood-borne carrying proteins, the corticosteroid-binding globulins (CBG) (Romero, 2004). A negative feedback signal shuts off the HPA pathway, leading to corticosterone release. If the stressor persists and corticosterone levels remain elevated, the negative feedback system becomes desensitised to glucocorticoids such as corticosterone and ceases to function. According to Siegel (1980) the changes that occur due to the production of corticosterone are commonly white blood count hyper-activation and functional changes, antibody suppression, increases in blood glucose and an increase in fat metabolism.

Leche *et al*. (2009) reported that corticosterone was the glucocorticoid present in Greater Rhea after an ACTH challenge. They injected birds intravenously with ACTH to determine if corticosterone was the glucocorticoid present in the plasma of Greater Rhea. They reported that there was an increase in the corticosterone concentration in the Greater Rhea's blood from baseline (time of injection) to 15, 30 and 60 minutes post ACTH administration. Baseline value were 3.98 ± 1.04 ng/ml compared to 89.83 ± 12.42, 141.6 ± 21.98 and 166.54 ± 16.01 ng/ml for 15, 30 and 60 minutes respectively, after ACTH administration. This constitutes an increase of almost 40-fold in corticosterone levels. They also reported that the corticosterone levels did not significantly differ from baseline values at 24 and 48 hours post ACTH administration, thus indicating recovery. They concluded that the magnitude of the adrenocortical response, seen after ACTH injection, suggests that the HPA axis of the ratite species was highly sensitive to stressful situations. The evidence reported by Leche *et al*. (2009) on corticosterone was also supported by Nagra *et al*. (1963), who reported a 250 % increase in corticosterone in various avian species after injection with ACTH.

The baseline values for plasma corticosterone in Greater Rhea (Leche *et al*., 2009) are similar to the ostrich (4.9 ± 2.9 ng/mL; Mitchell *et al*., 1996). A 75 % increase in plasma corticosterone ($P < 0.05$) was also observed when Mitchell *et al*. (1996) transported ostriches. The increase in plasma corticosterone concentration and in H:L ratio indicate that the HPA axis of the ostriches was triggered in response to a stressor, namely transport, and that corticosterone was the glucocorticoid released in ratites as an adaptive response to stress (Mitchell *et al*., 1996).

In a study to determine the effect of different capturing methods on stress levels in ostriches, Diverio *et al*. (2003) reported higher values of corticosterone in ostriches caught by a hook than that encountered in ostriches that were captured by luring them with food into a pen (also known as a passive capturing method). The Brown Kiwi also had a 50-fold increase in the concentration of corticosterone after they were captured and immobilised for 30 minutes (Leche *et al*., 2009).

2.2.6. Other measurable blood metabolites as indicators of *ante-mortem* stress

Stress caused by physical exertion during transportation can disrupt the homeostasis and metabolism of animals (Lopez *et al*., 2006; Averos *et al*., 2008). The physical exertion of transport causes an increased plasma concentration of enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK), as well as increases in other substances such as lactic acid, glucose and nitrogen urea (Parker *et al*., 2003b, 2007).

The haematological changes of adult (3-5 years) Red and Blue Neck ostriches, of both sexes before and after transport, are depicted in Table 2.13 (Minka & Ayo, 2008).

The heterophil counts of the ostriches increased significantly after transport by road over distances between 150 and 400 km, compared to counts before transportation (Minka & Ayo, 2008). On the other hand, the lymphocyte concentration decreased significantly after transportation. These changes in heterophil and lymphocyte values led to a significant increase in the H:L ratio of these ostriches (Minka & Ayo, 2008). As previously mentioned, an increase in H:L ratio in ostriches being transported is a good indication that transportation is a cause of stress to the birds.

Mitchell *et al*. (1996) conducted a trial on eight ostriches to determine the effect of transport stress on their blood chemistry values. Blood samples were taken from 10 month-old ostriches by venepuncture before and right after a 4.5 hour journey of 320 km. The internal temperature and humidity of the transport vehicle was 18.8 °C and 11.7 gm^{-3} throughout the trip and the birds were transported in a commercial livestock transporter that complied with normal standards. Various plasma concentrations of glucose, triglycerides, uric acid, non-estirified fatty acids (NEFA), lactate, protein, creatine kinase, aspartate aminotransferase and plasma corticosterone were measured and the heterophil:lymphocyte (H:L) ratio calculated (Table 2.14).

Table 2.13

Mean measurements (± s.d.) of the haematological variables of 50 ostriches, and the mean rectal temperature of 230 ostriches, before and after being transported by road (Minka & Ayo, 2008).

Blood metabolites	Unit	Before transport	After transport	P-value
PCV	l/L	0.68 ± 1	0.45 ± 0.7	P < 0.05
Haemoglobin	g/L	132 ± 20	158 ± 12	NS
Total RBC	× 10^{12} cells/L	6.8 ± 0.9	7.2 ± 1.1	NS
Total WBC	× 10^{9} cells/L	2.0 ± 0.2	2.4 ± 0.8	NS
Differential leukocyte count				
Lymphocytes	%	28.4 ± 2.7	19.7 ± 1.7	P < 0.05
Heterophils	%	60.1 ± 2.0	78.4 ± 2.4	P < 0.05
Monocytes	%	2.2 ± 0.7	2.8 ± 1.2	NS
Eosinophils	%	0.2 ± 0.1	0.5 ± 0.2	NS
Basophils	%	0.2 ± 0.3	0.4 ± 0.2	NS
Heterophil:lymphocyte ratio		2.12 ± 0.1	3.98 ± 0.2	P < 0.05
Rectal temperature	ºC	38.8 ± 1.2	40.5 ± 0.9	P < 0.05

Table 2.14

Plasma chemistry values of ostriches at 10 months of age, before and after a 4.5 h period of road transportation. Results are means (± s.d.; N = 8) (Mitchell *et al*., 1996).

Parameter	Unit	Pre-transport	Post-transport
Glucose	mmol/L	10.6 ± 0.93	18.5 ± 0.81
Lactate	mmol/L	11.6 ± 0.92	5.8 ± 0.3
NEFA	mmol/L	0.47 ± 0.05	0.73 ± 0.05
Triglycerides	mmol/L	0.81 ± 0.07	0.44 ± 0.02
Uric acid	mg/L	0.324 ± 0.077	0.853 ± 0.101
Protein	g/L	0.404 ± 0.007	0.469 ± 0.02
Creatine kinase	IU/L	560 ± 53	1398 ± 104
AST	IU/L	115 ± 6.7	137 ± 3.6
Corticosterone	ng/ml	4.9 ± 2.9	8.6 ± 3.7
H:L ratio		8.5 ± 3.2	24.2 ± 10.3

The results reported are summarised below (Mitchell *et al*., 1996):

A 1.7 fold increase in plasma glucose levels ($P < 0.01$)

A 50 % reduction in plasma lactate levels ($P < 0.01$)

A 55 % increase in NEFA ($P < 0.01$)

A 46 % decrease in triglycerides ($P < 0.01$)

A 2.6 fold increase in plasma uric acid ($P < 0.01$)

A 16 % increase in plasma protein ($P < 0.05$)

A tendency for the H:L ratio to increase

The increase in the H:L ratio indicates that the birds suffered stress, and this finding was underlined in video recordings obtained during transport that clearly showed birds exhibited aberrant behaviour such as stargazing, head bobbing and arching of the neck. The video recordings also showed that passing objects and cars upset the birds during transport, and that people approaching the truck while it was stationary, also evoked stressed reactions. Other physical signs of stress are commonly hyperventilation, fluffing of the wings and permanently keeping their beaks open (Wotten & Hewitt, 1999).

Kamau *et al*. (2002) conducted two trials on juvenile ostriches to determine the effect of mixing and translocating of ostriches into new groups and new environments, and the effect of corticosterone on lymphocyte and heterophil numbers. The ostriches were about 3 months old and weighed 18-20 kg. In the first trial to determine the effect of mixing and translocating birds, blood samples were taken from 15 birds 4 days and 2 days before mixing and translocation. Blood samples were again taken on days 2 and 4 after mixing and translocation (Table 2.15).

Table 2.15

The means (s.e.) of the percentage of heterophils, lymphocytes and H:L ratios in the blood of juvenile ostriches before and after mixing and translocating ostriches into new pens (Kamau *et al*., 2002)

	Pre-movement		Post-movement					
	Day -4		Day -2		Day +2		Day +4	
	Heterophil	Lymphocyte	Heterophil	Lymphocyte	Heterophil	Lymphocyte	Heterophil	Lymphocyte
Mean	21	79	27	73	35	65	45	54
SE	1.3	1.3	1.9	2	2.3	2.3	1.8	1.8
Min	17	66	20	60	17	49	30	45
Max	34	83	40	83	50	82	55	70
H:L ratio	0.27		0.37		0.53		0.83	

The mean H:L ratio increased ($P < 0.01$) from day 4 prior to movement to day 2 prior to movement. This change in H:L ratio is most likely caused by the handling of the birds by humans (for example during blood sampling), even though they had not yet been mixed and is an indication that the birds experienced stress.

After mixing and translocation, the H:L ratio of the birds increased ($P < 0.001$) from day 2 before mixing to day 2 after mixing, with an even bigger H:L ratio on day 4 after mixing and translocation.

In the second trial, Kamau *et al*. (2002) injected two ostriches with 1.5 ml Dexa 0.2 Phenix which contained 2.64 mg/ml dexamethasone, a synthetic glucocorticoid, to determine the effect of glucocortiocids on the concentrations of lymphocytes and heterophils in the blood of ostriches. Dexamethasone exerts an anti-inflammatory effect and is commonly used in avian medicines (Tully, 1995). Therefore it is not surprising that there was also a significant increase in the number of heterophils and a decrease in the number of lymphocytes after the administration of Dexa 0.2 Phenix (Table 2.16).

Table 2.16

The percentages of heterophils and lymphocytes in the blood of two ostriches on days 1 and 2, before administration of dexamethasone and on days 3 and 4 after administration. The H:L ratio on each day for each bird is also given (Kamau et al., 2002).

	Day 1		Day 2		Day 3		Day 4	
	Heterophil	Lymphocyte	Heterophil	Lymphocyte	Heterophil	Lymphocyte	Heterophil	Lymphocyte
Ostrich 1	23	77	22	78	44	56	45	55
Ostrich 2	21	79	27	73	48	52	42	58
Ostrich 1								
H:L	0.28		0.27		0.97		0.8	
Ostrich 2								
H:L	0.25		0.36		0.92		0.72	

These shifts in the numbers of heterophils and lymphocytes led to an increase in the H:L ratio of both the ostriches even until 4 days after injection. As already mentioned, the stress response of ostriches is mediated by the release of corticosterone in the blood. The stresses encountered during the first trial could have been from a number of factors. For instance, the change in the surface of the pens from concrete to sand, the breaking and forming of social bonds, a change in diet, change in handlers and a change in environment could all have played a role. All these stresses could have contributed to the increase in the H:L ratio of the birds in the first trial, according to Kamau *et al*. (2002) and confirms that stress in animals leads to an increase in H:L ratio.

Packed cell volume can be increased by splenic contraction, induced by catecholamine hormones, or by dehydration (Minka & Ayo, 2009). Dehydration and an increase in catecholamine hormones both occur during transportation. However, Minka and Ayo (2008) reported that the HCT decreased significantly (Table 2.15) after transportation. Some studies involving cattle report increased HCT after transportation (Tadich *et al*., 2005; Parker *et al*., 2007), while other studies by the same research teams show the opposite in sheep and cattle (Broom *et al*., 1996; Knowles *et al*., 1999a).

Plasma glucose is a very good physiological indicator of stress during transport (Knowles *et al*., 1999a; Tadich *et al*., 2005). The rise of plasma glucose concentration during transportation of animals is due to the breakdown of glycogen in the liver or skeletal muscles (Tadich *et al*., 2005). This process of glycogenolysis is fuelled by the release of glucocorticoids, such as corticosterone (Tadich *et al*., 2005). The increase in plasma glucose levels in the blood after transportation of ostriches reported by Mitchell *et al*. (1996) was probably connected to the fact that the lactate levels in the blood decreased after transportation (Table 2.14). The lactate probably acted as a substrate for the formation of glucose (Mitchell *et al*., 1996).

Erythrocytes count increased by 5.3 % in animals after transport (Minka & Ayo, 2009), which is probably a result of dehydration. If the amount of RBC in the blood would change after transport, it may well have an effect on the value of HCT in the blood of animals. In the study by Minka and Ayo (2008), there was also a slight but not significant increase in the RBC count of the ostriches after transport.

Leukocyte (white blood cell) count decreased by 3.9 % in animals after transport (Minka & Ayo, 2009). In the same study a decrease of 300 and 6.1 % in eosinophil and lymphocytes counts respectively, and an increase of 11.3 % in the neutrophil count were noted. According to Nwe *et al*. (1996), these changes in the blood cell counts of animals indicated dehydration. Guyton and Hall, (2006) proposed that glucocorticoids decrease the number of lymphocytes in the blood when the concentration of glucocorticoids is increased during stress. Guyton and Hall, (2006) further proposed that glucocorticoids reduces the negative effect of inflammation. However, still a lot of information is needed on the effect glucocorticoids have on the inflammatory response. Minka and Ayo (2008), however, found a slight though not significant increase in the concentration of total white blood cells in ostriches after transport. Minka and Ayo (2008) also reported that lymphocyte counts decreased after transport of ostriches, which agreed with earlier results (Minka & Ayo, 2009) in other animals. Minka and Ayo (2008) could, however, find no significant changes in basophil, eosinophil and monocyte counts as a result of transportation. Minka and Ayo (2008) did report that the heterophil count increased significantly after transportation, in agreement with Maxwell (1993). The decreased lymphocyte and increased heterophil counts reported by Minka and Ayo (2008) resulted in a significant increase in the H:L ratio of the birds after transportation, which also agreed with the earlier results by Maxwell (1993).

Parker *et al*. (2003b) observed that in cattle, plasma total protein and albumin increased after a long journey. He attributed these increases to dehydration (Parker *et al.*, 2003b). Similar results were also seen in pigs after transport (Brown *et al*., 1999a) and in ostriches by Mitchell *et al*. (1996) who reported a 16 % increase in plasma protein after transportation of ostriches. Minka and Ayo (2009) suggested that albumin is one of the major circulating anti-oxidants that protect cells against ROS (reactive oxygen species) by scavenging them. During times of environmental stress (such as exposure to heat), ROS levels can increase significantly and result in significant damage to cell structures – a phenomenon known as oxidative stress. Certain authors postulated that the increase in albumin during transport, is due to the response of the animal to the stressors encountered during transit (Krizanovic *et al*., 2008; Powers & Jackson, 2008). Damage to cell

structures can be minimised by supplementing ostriches with an anti-oxidant such as Vitamin E before transportation.

An increase in non-esterified fatty acids (NEFA) after long periods of food deprivation (e.g. during transport), is a good indication that body fat utilisation took place to supply the energy needs of the animal (Kannan *et al*., 2002). In studies conducted on pigs and cattle, NEFA increased significantly after transportation (Knowles *et al*., 1999a; Brown *et al*., 1999b). The increase in NEFA and the decrease in TG (triglycerides) in ostriches may indicate that the process of lipolysis was activated during transport, which in turn suggests that it could lead to the depletion of lipid stores to supply the energy needs of the animal during transport (Mitchell *et al*., 1996).

The concentrations of the enzymes aspartate aminotransferase (AST**)**, alanine aminotransferase (ALT) and creatine phosphokinase (CK), commonly increase in the blood of animals after transportation due to tissue damage and increased permeability of the muscle membrane for these enzymes (Warriss *et al*., 1995; Tadich *et al*., 2005). The animals suffer from fatigue during long periods of transportation, because they spend energy to maintain their balance on the truck. As already mentioned, it is hard for ostriches to maintain postural stability, because of their high centre of gravity, with sudden braking and acceleration aggravating the problem. This is why driver ability is of such importance during the transportation of ostriches. This act of balancing also causes bruises and physical exhaustion, and leads to increased permeability of membranes to enzymes, which are subsequently released into the blood (Brown *et al*., 1999a). Mitchell *et al*. (1996) reported that the concentration of CK and AST in the plasma of ostriches increased after transportation, supporting the view that during transport the integrity of the muscle cell membrane is altered and the concentrations of enzymes such as CK and ALT in the plasma, are increased. Diverio *et al*. (2003) studied different capturing techniques of ostriches and reported significant increases in the ALT, CK and LDH enzymes in plasma of ostriches that were captured by a hook, compared to the ostriches that were captured by food attraction into a pen. They suggested that the increases in these enzymes were probably due to physical exertion, after the forced restraining of the birds by hook.

2.3. PHYSICAL CHARACTERISTICS OF MEAT AS INFLUENCE BY *ANTE-MORTEM* STRESS

The physical characteristics of meat are the greatest factors affecting the decisions of consumers when they purchase meat products. Tenderness, colour and water-holding-capacity are the major physical characteristics of meat that are influenced by muscle pH in the conversion of muscle to meat (Lawrie, 1998). Muscle pH in turn is affected by both *ante-mortem* and *post-mortem* conditions. Prior to understanding the effect of *ante-mortem* stress on meat quality, a basic knowledge of the factors that influence the physical quality characteristics of meat is required.

2.3.1 Conversion of muscle to meat

When an animal dies, the circulation of blood through the animal's body ceases. This causes changes in the muscular tissue because the muscles are still continuing their particular type of metabolism. Energy is still required to maintain muscle temperature and organisational integrity during this early *post-mortem* period (Lawrie, 1998). ATP is required to keep the muscle from entering the phase of rigor and to keep the muscle relaxed during the *post-mortem* period. However, *post-mortem* muscle has a high ATP turnover rate which leads to the depletion of ATP. The death and subsequent bleeding of an animal eliminates and decreases the blood-borne oxygen supply to the muscles and ultimately leads to a fall in the oxidation reduction potential. The fall in the oxidation reduction potential leads to the cytochrome system's inability to resynthesise ATP (Lawrie, 1998). The available muscle glycogen in the muscles will then be metabolised through anaerobic glycolysis to rephosphorylate ADP to ATP, using creatine phosphate to prevent permanent actomyosin cross-links (Scheffler & Gerard, 2007). As the glycogen is broken down, it forms lactic acid that builds up in the muscles and decreases the muscle pH. However, this process cannot continue unabatedly due to the fact that glycogen stores become depleted *post-mortem*. This means that ATP production *post-mortem* stops after a while and this is when *rigor mortis* sets in (Lawrie, 1998). This also means that the pH of the meat can only decrease until a certain level. Warriss (2000) proposed that *post-mortem* pH falls in most animals are similar and that the pH fall tends to typically start at around a pH of 7 and end around a pH of 5.5. The onset of rigor mortis *post-mortem* varies between species and is influenced by the levels of muscle glycogen and creatine phosphate when the animal dies (Warriss, 2000). Swatland (1994) proposed that when ATP is completely depleted *post-mortem,* it signals the completion of the conversion of muscle to meat.

2.3.2 Tenderness

Tenderness of meat is described as "the ease of shearing or cutting during mastication" (Cooper & Horbanczuk, 2002). Tenderness of meat plays the biggest role in consumer preference for meat products (Risvik, 1994; Cooper & Horbanczuk, 2002). According to Sales and Oliver-Lyons (1996), there is little agreement between studies on the level of tenderness in ostrich meat. In a study by Harris *et al*. (1994) consumers rated the tenderness of ostrich meat similar to a choice beef loin steak.

There are three factors that influence meat tenderness, namely the intrinsic characteristics of the muscle, the toughening phase and the tenderisation phase. The intrinsic characteristics of the muscle refer to the quality and maturity of the connective tissue present in the muscle (Olssen *et al*., 1994). The two main types of connective tissue in meat are collagen and elastin. Collagen is a tissue that is straight and inextensible, whilst elastin is a tissue that is elastic and forms branches with other tissues within the meat (Lawrie, 1998). However, elastin forms only a minor part of the connective tissue, whilst collagen constitutes about 30 % of the total protein in mammals. Thus, although elastin toughens during heating, it plays a substantially lesser

role on meat toughness when compared to collagen. Collagen increases meat toughness due to the formation of non-reducible cross-links between collagen molecules (Davis *et al*., 1979). The cross-links of collagen molecules form a three-dimensional network that causes an increase in the tensile strength of the meat over long periods of time as the animal becomes older (Lawrie, 1998). This means that meat from older animals will most likely be tougher than meat from younger animals due to the formation of non-reducible collagen cross-links. Hoffman and Fisher (2001) observed that old ostriches had significantly higher shear force values compared to younger ostriches. To add to the problem of increased collagen that forms cross-links during ageing, a fraction of these cross-links is heat stable, meaning that these links will remain even after the meat has been cooked (Young & Gregory, 2001). This means that if these heat stable cross-links would increase in a muscle, it would lead to increased meat toughness.

The toughening and tenderisation phase of meat happens during the *post-mortem* period. The toughening phase usually happens within the first 12 hours *post-mortem* during rigor development when the sarcomeres of the muscles shorten. There is evidence to support the fact that sarcomere shortening is a cause of meat toughening (Koohmariae, 1996). ATP becomes depleted as the muscle enters rigor, and this result in the overlapping of actin and myosin. This leads to an increase in toughness. The degree of overlapping between actin and myosin can be influenced by temperature.

The tenderisation phase of muscle takes place at the same time as *rigor mortis*. The tenderisation phase is also known as *post-mortem* proteolysis. According to Fearson and Foster (1922), increases in meat tenderness *post-mortem* is associated with increases in water-soluble nitrogen. This indicates that there must have been some sort of protein breakdown in the muscles to peptides and amino acids. These changes that occur in the myofibrillar proteins *post-mortem* are initiated by the release of Ca^{2+} ions from the sarcoplasmic reticulum and altered by enzymes such as calpains. The calpains belong to a family of Ca^{2+}-dependant proteinases that proteolyze the protein calpastatin as meat pH drops below 6 and the levels of Ca^{2+} increases *post-mortem*. Another enzyme system also involved in the tenderisation phase of meat *post-mortem* is that of the proteolytic lysosomes; cathepsins with pH optima above 6 (Etherington, 1984).

The calpain system consists of three proteases namely μ-calpain, m-calpain and skeletal muscle-specific calpain. Along with the calpains there are also the calpain inhibitors called calpastatins. The calpastatins have helical sequences that prevent the calpains from binding to membranes. Calpastatin functions as a substrate to calpains, inhibiting the binding of calpains to membranes. When the pH of meat drops below 5.5 and the calcium levels increase, the calpastatin is proteolyzed by the calpains (Lawrie, 1998). Proteolysis by enzymes is also dependant on temperature. Proteolysis seems to be greater at higher temperatures (37°C) compared to lower temperatures (5°C).

There seems to be differences in tenderness between different muscles as well. The *M. iliofibularis* was the most tender ($P < 0.001$) whilst the *M. gastrocnemius* was the least tender ($P < 0.001$) (Pollok *et al*., 1997a; Girolami *et al*., 2003). These differences in levels of tenderness between various muscles can be attributed

to the different collagen content of the muscles. However, Sales and Mellet (1996) found no difference in tenderness when they compared the *M. iliofibularis, M iliotibialis lateralis and M femorotibialis medius.* In the study by Hoffman *et al*. (2008), no significant differences in tenderness could be found, when different genotypes of ostriches were compared.

2.3.3 Colour

Meat colour is one of the most important criteria when consumers select meat (Warriss, 2000). According to Ngapo *et al*. (2004) consumers prefer normal-coloured meat and mostly discriminate against meat that is either too pale or too dark. Consumers associated meat with a brown colour with contamination (Quali *et al*., 2006). The colour of meat is affected by various factors such as *post-mortem* pH fall, muscle-fibre type (Lawrie, 1998), myoglobin content (Sales, 1996) and the levels of intramuscular fat (Girolami *et al*., 2003).

The characteristic red colour of fresh meat is primarily caused by the myoglobin protein. The main function of myoglobin in muscles is to serve as a short term oxygen store during muscle contraction (Meyer, 2004). This means that the myoglobin content is higher in more aerobic muscles and muscles that have a more prolonged impedance of blood flow (and oxygen) during contractions (Meyer, 2004). Shorthose and Harris observed that free range animals have greater concentrations of myoglobin than animals that were raised in a feedlot. This is due to the higher cumulative exercise that the free range animals receive during their life.

The colour of raw ostrich meat varies from dark red to a slightly cherry red, when compared to the colour of beef that is mostly cherry red (Morris *et al*., 1995). This darker colour of ostrich meat is often discriminated against by consumers. According to Lawrie (1998), the amount of myoglobin is not the only factor affecting meat colour, but the type of myoglobin, its chemical form and other physical and chemical components of meat also influence this characteristic of meat. Oxymyoglobin is a chemical form of myoglobin present in fresh meat after the myoglobin protein has been exposed to oxygen. The ability of the iron in the porphyrin ring, present in myoglobin, to bind to oxygen results in the process of oxygenation. Oxygenation converts myoglobin to oxymyoglobin and leads the development of a bright cherry-red meat colour that is appealing to consumers (Mancini & Hunt, 2005). However, discoloration of meat can occur when the surface of the meat is exposed to the atmosphere for long periods. The discolouration (brown meat colour) is due to the oxidation of iron in the porphyrin ring from a ferrous to a ferric iron state (Mancini & Hunt, 2005). The formation of metmyoglobin also depends on numerous factors such as: oxygen partial pressure, low pH, high temperatures and meat's reducing activity (Mancini & Hunt, 2005). Meat colour is perceived differently among different consumers and it is therefore important to standardise colour observations. The CIELab colour scale (Commission International de L'Eclairage, 1976) as proposed by Honikel (1998) has been used in meat analysis trials to measure meat colour instrumentally. The CIELab colour scale has three measurements namely *L*, a** and *b** and the hue angle and chroma can be calculated from the three colour measurements. The *L** value indicates lightness, *a** value indicates the red-green range, the *b** value

indicates the blue-yellow range, whilst the hue angle and chroma values are an indication of colour definition and colour intensity respectively (Honikel, 1998). According to Sales and Oliver-Lyons (1996), fresh ostrich meat has an unique dark visual colour in comparison to beef. Like the meat of game species, ostrich meat is characterised by *L** values lower than 40, high *a** and low *b** values. In a study on young (14 months) and old ostriches (8 years) the CIELab values ranged from 29.42 ± 0.041 and 24.84 ± 0.574 for *L**, 5.48 ± 0.383 and 9.45 ± 0.541 for *a** and 3.51 ± 0.27 and 4.68 ± 0.382 for *b** measurements respectively (Hoffman & Fisher, 2001). When colour of different species and crossbreds of ostriches was analysed by Hoffman *et al*. (2008), no difference between the pure Blue, pure Black and crossbred Black and Blue ostriches in *L** values in the majority of the muscles was noted. The only exceptions were the higher *L** values (lighter colour) of the *M. fibularis longus* and *M. iliofibularis* of the African Black that was attributed to the slightly lower ultimate pH of the muscles from the African Black. Similar CIELab values for ostrich meat were also observed in an earlier study by Hoffman *et al*. (2005)

2.3.4 Water-holding capacity

The term 'water-holding capacity' (WHC) is described as 'the ability of meat to retain water during the application of external forces' such as cutting and heating (Swatland, 1994). According to Offer and Trinick (1983), fresh meat consists out of about 75% water. The majority of water in meat is found within the myofibrils between the thick and thin filament space (Offer & Trinick, 1983). This water can either be classified as bound, entrapped or free. Bound water is found within the filament where it is associated by a hydrogen bonding to myofibrillar proteins whilst entrapped water is the fraction of water molecules that is held by steric effects and/or by attraction to the bound water fraction within the structure of the muscle (Huff-Lonergan & Lonergan, 2005). Entrapped water is not bound to proteins like bound water and during the rigor process it is this entrapped water that is affected the most during the conversion of muscle to meat (Huff-Lonergan & Lonergan, 2005).

According to Lawrie (1998) the interfilament spacing (between the thick and thin filaments) mainly determines the WHC of the meat. The expansion or contraction of filaments determines whether there will be uptake or expulsion of water in the muscles and these changes in the filaments are due to the interaction of actin and myosin (Warriss, 2000). The thin filaments are made up primarily of actin, whilst the thick filaments are made up of myosin. The actin and myosin from each filament interact to form the actomyosin complex or rigor complex (Huff-Lonergan & Lonergan, 2005). When muscle is converted to meat, there is a reduction in pH of the meat due to lactic acid built up in the muscle tissue during *post-mortem* anaerobic glycolysis. As pH decreases during the *post-mortem* period, the meat reaches its iso-electric point where major proteins such as myosin have a net charge of zero. This means that the total number of positive and negative charges on the proteins is essentially equal. Positive and negative charges within proteins are attracted to each other and this will result in a reduction in the amount of water that can be attracted and held by the protein. On the other hand, like charges repel. This means that as the net charge of the proteins in the

muscle approaches zero, there will not be any repulsion of charges within proteins, allowing the structures within the myofibril to pack more closely (Huff-Lonergan & Lonergan, 2005). This result in a reduction of space within the myofibril at the iso-electric point of meat and causes the lattice to shrink and expel water from the meat (Warriss, 2000; Huff-Lonergan & Lonergan, 2005). The water expelled to the extracellular space is commonly known as purge or exudate. Purge formation in meat packaging leads to meat products that are unacceptable to consumers, whilst purge losses also result in lower meat weights and have a negative effect on income. Thus WHC is mostly influenced by meat pH. However, WHC tends to be higher for a muscle that has increased intramuscular fat, possibly due to the fat loosening the muscle's microstructure and retaining more water (Harris *et al*., 1994). Harris *et al*. (1994) also observed that ostrich steaks were much drier than beef loin steaks, the latter having higher intramuscular fat levels. Sales and Horbanczuk (1998) proposed that the WHC of meat has the ability to influence other meat characteristics such as juiciness, colour and tenderness.

Drip loss or purge can be calculated during meat analysis and is often used as an indicator of meat quality. Cooking loss is also a measure of WHC in meat, and in conjunction with drip loss is considered to be indicators of meat quality. During cooking, meat is subjected to high temperatures that cause denaturation of proteins and disrupt the cellular structures, leading to water being released from the meat (Honikel, 2004). According to Lawrie (1998), cooking loss is influenced by the temperature, duration and time of cooking. For instance, at temperatures higher than 64°C, collagen shrinks in the endomesium and perimeseum which can affect cooking loss values if compared to other studies with lower cooking temperatures (Sims & Bailey, 1981).

2.3.5 The relationship between pH and the physical characteristics of meat

Anaerobic glycolysis of glycogen *post-mortem* leads to the accumulation of lactic acid in the muscles. The acidification of the muscles by lactic acid is measured in terms of pH. The rate and extent of acidification (that influences ultimate pH) has the ability to influence meat characteristics such as colour, water-holding capacity and tenderness. The decline of pH can be best described by an exponential decay curve (Bruce *et al*., 2001) and is presented in Figure 1. When muscle pH declines *post-mortem*, the muscle proteins denature and lead to the shrinking of the myofilament lattice. This leads to an increase in the myofibrillar index that eventually exceeds that of the sarcoplasm and increases light scattering. The increased light scattering decreases the effect of myoglobin and leads to meat being lighter (Swatland, 2004). According to Swatland (2004) meat with low pH are also pale compared to meat with higher pH. Guignot *et al*. (1994) found a negative correlation between the meat colour measurements of lightness, redness and reflectance when correlated to ultimate pH (pH_u). This means that meat with higher pH exhibited darker coloured meat and *vice versa*.

As pH decreases during the *post-mortem* period, the meat reaches its iso-electric point. The drop in meat pH, *post-mortem* to its iso-electric point, also coincides with a decrease in the WHC of the meat at a pH around 5.0 to 5.5 (Swatland, 1994). According to Hoffman *et al*. (2005) the WHC of ostrich meat tends to be stronger if the pH_u is higher, because the filament fibres will be tightly packed together, creating a barrier for the diffusion of water. The findings of Hoffman *et al*. (2005) agree with that of Fasone *et al*. (2005) who found that meat with a high pH had significantly less cooking loss. Guignot *et al*. (1994) also found a negative correlation between drip and cooking loss percentages when they correlated these losses to ultimate pH. Offer and Knight (1988) postulated that high temperatures and low ultimate pHs could cause protein denaturation *post-mortem* and lead to a decrease in the WHC of meat. WHC is mostly influenced by meat pH. However, WHC tends to be higher for a muscle that has increased intramuscular fat, possibly due to the fat loosening the muscle's microstructure and retaining more water (Harris *et al*., 1994). They observed that ostrich steaks were much drier than beef loin steaks, the latter having higher intramuscular fat levels.

Although many factors influence tenderness of meat, pH also has an impact on this characteristic of meat. According to Purchas (1990), there seems to be a curvilinear relationship between the ultimate pH and the tenderness of meat, with tenderness decreasing as the pH_u increases from 5.5 to 6.0, whilst meat with pH_u >6.0 become more tender as the pH_u increases to 7.0. Yu and Lee (1986) attributed the increased tenderness of the meat at high pH_us (pH_u > 6) to the greater calpain activity in meat at a neutral pH, *post-mortem*. They concluded that the increased tenderness observed at low pH_us (pH_u < 5.5) be attributed to greater cathepsin activity at an acidic pH_u. Yu and Lee (1986) postulated that the decreased tenderness observed in meat with a pH between 5.8 and 6.2, be attributed to the fact that at this range of pH the two main proteolytic enzyme systems didn't function at their optimum pH. Another hypothesis postulated by Purchas (1990) was the influence that pH had on sarcomere length and the influence sarcomere length in turn had on tenderness. However, it seems that the extent of *post-mortem* glycolysis has the greatest effect on pH, leading to pH conditions that are either optimal for certain protease enzyme systems or not, during the tenderisation process.

2.3.6 The effect of stress on muscle pH *post-mortem* and its implications for meat quality

Meat quality is influenced by a number of factors, but the acidification of meat *post-mortem* seems to the most crucial factor. The amount of glycogen available in the muscles for post-mortem glycolysis will determine the rate and extent of pH decline during this period (Lawrie, 1998). The rate and extent of pH decline depends on factors such as species, muscle and nutritional status of the bird. However, it seems as if *ante-mortem* stress plays a major role in determining the amount of glycogen present in muscles prior to slaughter. According to Sales and Mellet (1996), *ante-mortem* stress leads to glycogen depletion prior to slaughter, which in turn leads to lower lactic acid production during the *post-mortem* period. Insufficient lactic acid production in muscles *post-mortem* leads to the production of meat with a high ultimate pH. This meat is

also known as dark firm dry (DFD) meat. As previously mentioned, the glucocorticoid hormone corticosterone is released in the blood of avians when they encounter a stressful situation and a high level of free corticosterone is maintained in the blood during a prolonged stress response (Leche *et al*., 2009). When in danger, ratites need sufficient glucose to enable them to use their fast-running ability as an anti-predatory strategy. This glucose is made available by the hyperglycaemic effect of corticosterone to aid the animal in a period when it requires sufficient energy for a flight-type stress response (Leche *et al*., 2009). The glycogen reserves of the animal becomes depleted due to *ante-mortem* stress and ultimately leads to meat with a high pH or in extreme cases DFD meat *post-mortem*.

Fasone *et al*. (2005) reported that the ultimate pH of birds that were subjected to stress, compared to birds that were not subjected to stress, was significantly ($P < 0.05$) higher. The stressed group's average ultimate pH was 6.95 ± 0.17, compared to the control group that had an average ultimate pH of 5.94 ± 0.08 (Table 2.17). It must be noted that the birds that were stressed, were severely stressed due to reasons beyond the control of the producers and included conditions such as broken legs and insufficient stunning. DFD meat has been a major concern for meat producers due to its poor processing characteristics, as well as its unacceptability to meat consumers (Warriss, 2000). Figure 2.1 represents patterns of acidification of DFD, PSE (pale soft exudative) (a condition that isn't seen in ostrich meat) and normal meat.

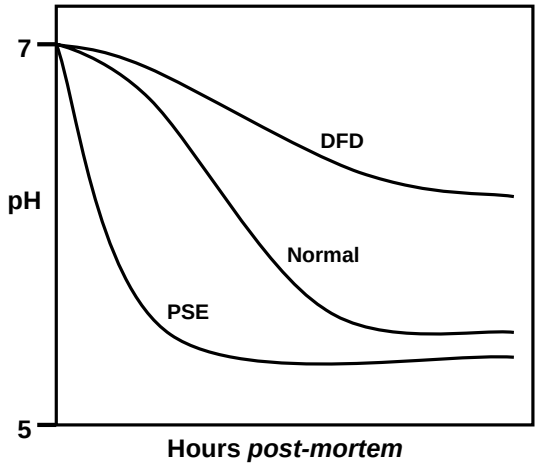

Figure 2.1 The pattern of acidification in PSE, DFD and normal meat illustrated schematically (adapted from Warriss, 2000).

The pH of living muscle is 7.4 (Briskey & Wismer-Pederson, 1961). Sales and Mellett (1996) found that the ultimate pH of ostrich meat is reached 2-6 hours *post-mortem* in various muscles. They classified the pH of ostrich meat as intermediate, with the pH values normally lying between normal (pH < 5.8) and DFD meat (pH > 6.2). This means that the pH of ostrich meat has a rapid decline from 7.4 to about 5.8-6.2 within 6 hours *post-mortem*, regardless of factors such as electrical stimulation and *ante-mortem* conditions (Hoffman

et al., 2009). In other animals such as pigs (8-12 hours), sheep (24 hours) and cattle (36-48 hours) ultimate pH is reached at later stages *post-mortem* (Forrest *et al*., 1975).

As previously mentioned, chronic stress during the *ante-mortem* period of animals can lead to DFD meat. According to Sales and Mellett (1996) DFD ostrich meat would exhibit pH values above 6.2. The depletion of glycogen reserves, before slaughter, leads to limited *post-mortem* anaerobic glycolysis which in turn leads to a decrease in the build-up of lactic acid in the muscles and ultimate increased meat pH. The higher-than-normal pH of DFD meat leads to a decrease in protein denaturation and causes the water molecules to bind more tightly to the muscle proteins (Guignot *et al*., 1993). This increases the WHC of the meat, as was also underlined by Fasone *et al*. (2005). Fasone *et al*. (2005) reported a lower average percentage cooking loss in meat of stressed ostriches, compared to meat of ostriches that were not stressed. The values for stressed birds and the control group of birds were 19.46 % and 23.46 % respectively ($P < 0.01$). These values were in accordance with Hoffman and Carbajo (2005), who suggested that the shorter sarcomers of DFD meat could explain the increased WHC of the stressed ostrich's meat. In the study of Fasone *et al*. (2005) the stressed group of birds suffered from (acute) stress that was beyond the control of the producer, such as a broken leg during transit or insufficient stunning (Table 2.17). During mastication, DFD meat keeps water molecules so tightly bound to protein molecules that the meat is perceived as dry. WHC also affects meat colour, as a higher WHC results in a higher water content of the meat, thus causing a higher absorption of radiation and lower reflection, ultimate leading to the meat being darker (Balog & Almeida Paz, 2007). Fasone *et al*. (2005) found significant differences in meat lightness (L*) due to stress ($P < 0.001$) compared to the control group of birds (stressed group 34.30 vs 38.10). Sales and Mellet, (1996) also found lower L* values in stressed birds and suggested that it is the cause of the higher ultimate pH. Fasone *et al*. (2005) found no differences between other colour parameters such as Redness (a*), Yellowness (b*), chroma and hue angle.

The dark colour of DFD meat often leads to it being discriminated against by consumers, as previously mentioned. However, the appearance of DFD meat isn't the only problem that supermarkets have to face when selling DFD meat. DFD meat also has a shorter shelf life than normal meat. The effect ultimate pH has on shelf life is due to the interaction of meat pH and microbiological quality/growth. An ultimate pH value of 6 and more causes favourable conditions for the proliferation of microorganisms that cause foul odours in meat (Balog & Almeida Paz, 2007). As the time spent on the shelf increases, this can lead to an increase in bacterial load, consequently reducing the shelf life of the ostrich product (Balog & Almeida Paz, 2007). According to Pollok *et al*. (1997a), the microbiological quality of ostrich fillets stored under refrigeration was impaired after 14 days, and after 21 days the anaerobe plate cultures exceeded 6 $\log/cm^2$, which made the fillets unfit for human consumption. DFD meat is a major problem in ostrich meat regardless of *ante-mortem* stress and it seems as if high ultimate pHs (pH_us) are a unique characteristic of the ratite species (Hoffman *et al*., 2007). However, it would appear as if the herding, transport and handling of these birds aggravate the problem of DFD meat in South Africa.

There does not seem to be consensus on the effect pH_u has on the tenderness of meat. Fasone *et al*. (2005) found a significant difference in the Warner-Bratzler shear (WBS) force values, when they compared stressed and unstressed ostriches, pre-slaughter (Table 2.17).

Table 2.17
The mean (± s.d.) values of the instrumental measurements of ostrich meat samples of a stressed group and a control group (no stress) (Fasone *et al*., 2005).

Parameters	Stress group	Control group	P - value
Physical variables of meat at 24 h post-mortem (Muscle *iliofibularis*)			
pH	6.95 ± 0.17	5.94 ± 0.08	P < 0.001
Lightness (L*)	34.3 ± 1.9	38.1 ± 2.21	P < 0.001
Redness (a*)	14.56 ± 1.52	14.92 ± 1.67	NS
Yellowness (b*)	4.49 ± 1.24	4.85 ± 1.28	NS
Chroma	15.14 ± 1.62	15.7 ± 1.95	NS
Hue angle	16.77 ± 2.74	17.74 ± 2.99	NS
Cooking loss (%)	19.46 ± 4.97	23.46 ± 3.86	P < 0.001
WBS (kg Fcm^{-2})	6.13 ± 2.05	7.11 ± 1.72	P < 0.01

2.4. Other intrinsic factors affecting meat quality

2.4.1 Age

As the age of an animal increases, the muscle composition of the animal will also start to vary, irrespective of the sex, species or breed of the animal. Research conducted on various species indicated common trends in muscle composition variability with age (Lawrie, 1998).
An increase in age was accompanied by an:

- increased saturation of intramuscular lipids
- increased toughness of the meat due to the increased connective tissue in the muscle
- increased myoglobin concentration in muscles
- increased intramuscular fat content

One of the most notable parameters of meat, influenced by age, is tenderness. This is due to the changes that occur in the connective tissue as the birds age. With age, the intramuscular connective tissue of the ostrich becomes more stable due to an increase in the total of heat stable cross links in the collagen (Light, 1987). Aberle *et al*. (2001) proposed that after the growth phase of an animal is completed, there is an increase in these collagen fibre cross links, accompanied by increased toughening of the meat. The collagen

content in ostrich meat is low (0.41 %) when compared to beef (0.61 %) (Sales & Oliver-Lyons, 1996). When Hoffman and Fisher (2001) compared shear values of old birds to results of Sales (1994) on younger birds (8 months), they found that shear values were significantly higher for the old ostriches. In the emu and chicken, similar results were found (Berge *et al*., 1997). However, Mellett and Sales (1997) could not find any differences in Warner- Bratzler shear force values when they compared shear values of birds slaughtered at 8, 10, 12 and 14 months respectively. This finding was supported by Girolami *et al*. (2003) who compared ostriches slaughtered at 10-11 and 14-15 months of age. The above findings could indicate that only extreme differences in ages of ostriches at the time of slaughter could affect tenderness perceived by consumers.

Hoffman and Fisher (2001) reported that ostrich meat becomes significantly darker and more red with an increase in age when they slaughtered ostriches at an age of 8 years and compared their results to studies done by Horbanczuk and Sales (1998) and Sales (1998). These differences in colour with age were also observed in the emu, chicken, pork and beef (Berge *et al*., 1997; Lawrie, 1991). The differences seen in colour are probably due to the increases in concentration of the pigment myoglobin in meat of older animals (Lawrie, 1998). Even though the meat becomes darker with age, it has to be remembered that regardless of age and muscle type analysed, the ostrich and other ratites have a significant, intense red colour due to their high pigment content (Berge *et al*., 1997; Sales, 1996).

When Hoffman and Fisher (2001) compared ostriches of 8 months and 8 years to each other, they found no significant differences in cooking loss (water-holding-capacity) between these two age groups.

The meat of younger ostriches generally contains less fat than older birds (Hoffman & Fisher, 2001). However, Sabbioni *et al*. (2003) found no difference between the fat content of ostriches between 10 and 54 months of age, but they found a linear increase in the protein content of muscles. They also observed that the *M. iliofibularis longus* muscle's fat quality was affected by an increase in the age of the birds. The saturated:unsaturated fatty acid ratio increased with an increase in the age of the ostriches. In general, older animals contain a higher percentage saturated fatty acids (SFA) and less polyunsaturated fatty acids (PUFA) than younger animals (Lawrie, 1998). This fact was underlined in a study by Girolami *et al*. (2003), when they found significant differences in the fatty acid composition of Blue Neck ostriches slaughtered at 10-11 and 14-15 months of age. The increasing slaughter age was accompanied by an increase in monounsaturated ($P < 0.001$) and total saturated ($P < 0.005$) fatty acids and a decrease in PUFA ($P < 0.001$). The changes in the fatty acid profile of meat from ostriches can also be influenced by the type of diet, as well as the total fat content of the muscle, with older birds having a greater total muscle fat content that younger birds.

There were no differences in the cholesterol content of birds slaughtered at different ages (Girolami *et al*., 2003). This finding is supported by Horbanczuk *et al*. (1998) who also found no difference in cholesterol content between individual muscles.

Girolami *et al*. (2003) found no differences in the flavour intensity of meat from ostriches slaughtered at 10-11 and 14-15 months of age as perceived by a sensory panel. Pollok *et al*. (1997) also found no differences in meat flavour intensity when they slaughtered ostriches of different ages.

2.4.2. Species and sub-species

Specie differences have the ability to influence factors of meat quality such as muscle composition, muscle growth and muscle activity. Less-domesticated animals normally perform a greater amount of exercise. Greater muscle activity leads to an increased amount of myoglobin in the muscles, which in turn leads to darker meat (Lawrie, 1998). Ostrich meat is extremely lean in comparison to beef and has very low levels of intramuscular fat (on average 2.3 %) of which 66 % is unsaturated fat that is easily digestible (Sales, 1996; Paleari *et al*., 1998). The leanness of the ostrich carcass is mostly due to the fact that the majority of fats are present subcutaneously and in the cavities of the carcass (Cooper & Horbanczuk, 2002). This makes the fat easily separable during processing (Cooper & Horbanczuk, 2002).

Juiciness, tenderness and flavour are common sensory properties, perceived by the consumers of meat. Unlike beef, it was observed by taste panels that ostrich meat has a unique aftertaste (Harris *et al*., 1993; Paleari *et al*., 1995). The taste panels described the ostrich meat as bland. According to Lawrie (1998) and Cooper and Horbanczuk (2002) the blandness taste experienced could be attributed to the high ultimate pH and low intramuscular fat content of ostrich meat. Juiciness of meat is directly attributed to water-holding capacity of the meat (Offer & Trinick, 1983). Another factor that can also affect juiciness is the fat content of the meat. Fat stimulates the secretion of saliva during eating and contributes to improved juiciness (Lawrie, 1998). The low intra-muscular fat content of ostrich meat (Sales, 1995) coupled with the high ultimate pH, that causes a stronger water-holding capacity and DFD meat (Hoffman, 1988) is the main reasons why ostrich meat was perceived as less juicy, when compared to lamb and pork by taste panels (Rodbottem *et al*., 2004). Hoffman *et al*. (2008) compared various physical and sensory characteristics of meat from purebred Blue, purebred South African Black and crossbred South African Black and Blue ostriches, to determine the effect sub-species has on meat characteristics. Hoffman *et al*. (2008) reported that the muscle pH after 24 hours for purebred Blue Necks was the highest, that they had the darkest muscles and lowest drip and cooking loss compared to the South African Black and a crossbred between the Blue and the South African Black ostriches. The reason for the higher pH_{24} in purebred Blue ostriches is that these birds characteristically become more nervous during handling in the *ante-mortem* period than the South African Black (Hoffman *et al*., 2008).

Hoffman *et al*. (2008) also determined the effect of species on drip loss and reported that two thirds of muscles analysed from the South African Black had more drip loss ($P < 0.05$) compared to the same muscles of the pure Blue sub-species. The lesser drip loss seen in most of the muscles of the pure Blue

birds is probably due to the pure Blue's higher pH_{24}. As the pH_{24} of the meat increased, there was a slight decrease in drip loss. In terms of cooking loss, the African Black, compared to the pure Blue, showed similar results to the above-mentioned drip loss results. In 50 % of the muscles analysed, the South African Black had a higher percentage of cooking loss (Hoffman *et al*. 2008). The lower pH_{24} of the muscles from the South African Black would lead to a lower WHC (Swatland, 1994).

2.4.3. Gender

It seems as if different sexes have different levels of fat deposition. Diaz *et al*. (2003) proposed that the differences seen in fat deposition between sexes be attributed to variations in the efficiency of protein assimilation and composition of weight gain between different sexes in lambs. Ostrich sex had no significant impact on meat yield according to Cooper and Horbanczuk (2002), however they observed a slightly higher percentage of carcass fat on female birds. On the other hand, Sabbioni *et al*. (2003) found no differences in chemical composition or fatty acid content of meat from different sexes. However, Sabbioni *et al*. (2003) reported that the shear force values were slightly higher for male birds. According to Sales (1994) and Jones *et al*. (1994), sex had no effect on tenderness in ostriches of the same age.

2.5. The effects of transport and lairage on weight loss and muscle yield

Transport of animals for long distances has been found to cause a reduction in live weight as well as in carcass weight (Minka & Ayo, 2007b; Minka & Ayo, 2008; Minka & Ayo, 2009). Weight loss during these periods result in economic losses to producers. Many farmers are of the opinion that ostriches lose more weight when they travel longer distances to an abattoir, regardless of the time they are deprived of feed. No studies could be sourced on the effect transportation distances has on live weight loss in ostriches. However, lairage also seems to have an effect on live weight losses in ostriches. According to Sabbioni *et al*. (2003) lairage times between 2-26 hours had a significant effect on ostrich carcass weight. In South Africa it is common practise to keep birds for 12-24 hours prior to slaughter at abattoirs with no food, but an adequate supply of water. Van Schalkwyk *et al*. (2005) compared the live weight change of birds that were kept for 12-24 hours in lairage to birds that were kept for 56 hours (stressed group – this time period represented lairage over a weekend) in lairage (Table 2.18).

Table 2.18

Means (± s.d.) for initial live weight, slaughter age, live weight change and drumstick weight of slaughter ostriches in relation to stress treatment (withdrawal of food for 2.5 days) or control (feed withdrawal for 24 h) (Van Schalkwyk *et al*., 2005).

Trait	Treatment		P-value
	Control	Stressed	
Number of observations	46	38	
Slaughter age (days)	271 ± 6	281 ± 7	NS
Live weight traits (kg)			
Initial live weight	76.9 ± 2.2	78.8 ± 2.4	NS
Live weight change	(-)1.04 ± 0.51	(-)3.23 ± 0.005	P < 0.005
Drumstick weight traits			
Number of observations	25	22	
Drumstick weight (kg)			
Hot	29.3 ± 1.0	29.1 ± 1.1	NS
After 26.5 hours at 2ºC	28.4 ± 1.0	28.5 ± 1.1	NS
Relative to initial live weight (%)			
Hot	35.7 ± 0.5	36.1 ± 0.6	NS
After 24 hours	34.7 ± 0.5	35.4 ± 0.5	NS

There was a significant weight change in the birds that were kept for 56 hours in lairage. They lost 3.23 ± 0.56 kg, compared to the group of birds that were kept in lairage for 12-24 hours, who lost 1.04 ± 0.51 kg (Van Schalkwyk *et al*., 2005). In the same study, no significant differences between the hot and cold drumstick weights of the two groups of ostriches were reported. This may indicate that a longer lairage time may cause losses in faecal content of the digestive system, but that lairage time did not significantly impact on muscle weight. However, to determine if there was a significant difference in digestive system weights, Van Schalkwyk *et al*. (2005) weighed the stomach and alimentary tract. Van Schalkwyk *et al*. (2005), however, found no differences in stomach and alimentary tract weights between the two groups. The reason for the difference in live weights of the two groups could not be explained. According to Sales and Oliver-Lyons (1996), ostriches have an excessive weight loss of between 10 and 17 % when they are transported and kept in lairage for 18-24 hours prior to slaughter.

Kannan *et al*. (2000) reported live weight shrinkage of 10 % when goats were transported for 2.5 hours and held in lairage for 18 hours. Similar effects on weight loss were also reported by Tarrant *et al*. (1992) on cattle. Kannan *et al*. (2007) used a seaweed extract as supplementation before transportation of goats, but

could not find any effect on reducing weight loss. Schaefer *et al*. (1990) reported that transport and handling cause significant changes in the electrolyte balance of animals, particularly the major ions such as potassium, magnesium, chloride and calcium. Schaefer *et al*. (1997) used electrolyte therapy during pre-transport in steers, and were able to reduce losses in carcass weight whilst Schaefer *et al*. (1993) used an electrolyte treatment before transport of steers and heifers and reported a significantly lower loss in live weight.

No studies could be sourced that reported on strategies to decrease weight loss during lairage and transportation in ostriches.

2.6. Bruising on carcasses during transport

The loading and offloading, and transportation of animals are the major contributing factors to bruises on carcasses (Grandin, 1990). McEwen and Barbut (1992) found a positive correlation between transport time and the incidence of carcass lesions. According to Hallam (1992), as cited by Wotten and Hewitt (1999), ostriches bruise easily and care has to be taken during handling to minimise bruising. Other damages on ostrich carcasses are dislocated and broken hips, internal haemorrhage, broken legs and broken wings. Due to the bipedal nature of ostriches and their high centre of gravity, ostriches struggle to keep their balance whilst in transit (Wotten & Hewitt, 1999). However there are a lot of other factors that can also affect balance during transport such as floor type, stocking density, driver ability and road conditions. This causes ostriches to fall or sit during transport and often leads to them being trampled. The trampling of birds can lead to serious damage to ostrich skins and meat and also negatively affects the welfare of the birds (Wotten & Sparrey, 2002). Poor handling and transportation can also lead to a traumatised bird and ultimately to increased mortality (Wotten & Hewitt, 1999). According to the FAO (Food and Agricultural Organisation), bruised meat is wasted because it is “aesthetically” unacceptable to consumers and spoils more rapidly due to the fact that the bloody meat is an ideal growth medium for bacteria during storage (Chambers *et al*., 2004). In South Africa, as a precautionary measure, when birds arrive at an abattoir with open wounds, they are returned to the farm for their wounds to heal.

Primary meat inspection on carcasses takes place in export abattoirs by a meat inspector of the Department of Agriculture, within one hour after slaughter (Anonymous, 2004). During the meat inspection, the inspector has to trim away all the bruises on the hot carcass. According to Hoffman *et al*. (2010), warm trimming of carcasses can lead to average losses of 300g per bird. Hoffman *et al*. (2010) found that the most prominent areas of bruises were the neck and thighs. They believed that if there was multiple bruising over the body, it was probably due to trampling. They proposed that cold trimming could reduce the losses in meat yield and that cold trimming could lead to a reduction in the microbial load of primary meat, increasing the shelf life of these meat cuts.

Damage to ostrich skin, such as bruises, kicking marks and wounds, leads to the skin being downgraded at the ostrich tannery (Wotten & Sparrey, 2002). This causes major economical losses due to the high economical value (30 % of total income per bird) of ostrich skin for the production of leather products.

2.7. Stress during lairage

Prior to slaughter, birds are usually kept in holding pens for lairage after transport. When periods of feed deprival during lairage exceed 24 hours, it can lead to increased pre-slaughter stress (Sales & Mellett, 1996). Most abattoirs in South Africa now provide feed to birds that remains in lairage for more than 24 hours. Lairage *per se* also causes stress due to other factors such as new flooring, higher penning density of birds, other flocks and unknown noises (Hoffman & Lambrechts, 2010). According to Hoffman and Lambrechts (2010) the objective of lairage, prior to slaughter, is to allow the animal sufficient time to restore their muscle glycogen levels, thereby allowing normal anaerobic muscle metabolism after slaughter that ensures good quality meat. Most slaughter animals are not kept for more than 24 hours in lairage and Hoffman and Lambrechts (2010) believed that this time is insufficient to restore muscle glycogen levels in red meat.

Van Schalkwyk *et al*. (2005) withheld feed from ostriches for approximately 56 hours (stressed group) and compared their results to a control group of birds that was deprived of feed for only 24 hours, prior to slaughter. Fifty-six hours is normally the lairage time ostriches are kept when they arrive at the abattoir over a weekend or on a Friday afternoon. Cooking loss, drip loss, shear strength and ultimate pH were some of the meat characteristics analysed on the *M. iliofibularis longus* (Table 2.19).

Table 2.19

Means (± s.d.) for meat quality parameters of ostrich fillets (*M. iliofibularis*) in relation to stress treatment (Van Schalkwyk *et al*., 2005).

Trait	Treatment		P-value
	Control	Stressed	
Number of observations	25	22	
pH			
1 h post-slaughter	5.81 ± 0.05	6.03 ± 0.06	P < 0.01
26.5 h post-slaughter	6.21 ± 0.07	6.46 ± 0.07	P < 0.05
Temperature (ºC)			
1 h post-slaughter	38.95 ± 0.44	39.84 ± 0.47	NS
26.5 h post-slaughter	2.19 ± 0.07	2.13 ± 0.08	NS
Cooking loss (%)	30.8 ± 1.2	29.1 ± 1.3	NS
Drip loss (%)	2.18 ± 0.24	1.88 ± 0.26	NS
Shear force (N)	0.078 ± 0.006	0.067 ± 0.007	NS

The intra-muscular pH of the stressed group of ostriches was higher ($P < 0.01$) than those in the control group 1 hour after slaughter, whilst at 26.5 hours after slaughter the stressed birds still had a higher pH ($P < 0.05$) than the control group (Van Schalkwyk *et al.*, 2005). This would indicate that the stressed birds had less muscle glycogen left for anaerobic glycolysis. There was also no significant difference between the stressed group of birds, compared to the control group on the basis of cooking loss, drip loss and shear strength (Van Schalkwyk *et al.*, 2005).

The rapid decrease in muscle pH after slaughter, with a high ultimate pH (not much below 6.0) was also observed by Mellett (1985) for ostriches. They concluded that this characteristic of ostrich meat led to moderate DFD meat, with a shorter shelf-life, an unacceptable dark colour for consumers and a low salt penetration for cured products (Lawrie, 1998). The shorter shelf life of DFD meat can be explained by the fact that glycogen is depleted in the muscles and this is characterised by very low levels of carbohydrates in the muscles. The low levels of carbohydrates, in turn prevents the growth of lactic acid-producing bacteria. The inferior growth of lactic acid bacteria encourages the growth of bacteria that metabolises protein that then produces unpleasant odours and waste products faster (Warriss, 2000). The high ultimate pH in the studies on ostriches, especially the study by Van Schalkwyk *et al*. (2005), who deprived ostriches of food for 56 hours, is an indication that a feed deprival period for a prolonged period during lairage, leads to a

decrease in glycogen reserves of the muscles and results in a higher ultimate pH. Although it is now unacceptable in South Africa to maintain ostriches in lairage for periods longer than 24 hrs without food, the long distances (>1000 km) that ostriches sometimes have to travel combined with a standard lairage period (12 - 24 hrs) would result in a cumulative period without feed of > 48 hrs. It is therefore probable that meat quality attributes similar to that of Van Schalkwyk *et al*. (2005) could be encountered. However it is very unlikely that birds will travel more than a 1000 km to a abattoir.

Dehydration during lairage also seems to be a problem. The *M. iliofibularis longus* fat content increased during lairage and this can be attributed to dehydration caused by stress (Sabbioni *et al*., 2003). The lairage period also has an influence on the fatty acid ratio and decreases the proportion of SFA and PUFA in the *M. fibularis longus* (Sabbioni *et al*., 2003).

As mentioned previously, there are noises and other activities that occur at an abattoir which are also stressful to a bird. Hoffman and Lambrechts (2010) believe that stresses commonly encountered during lairage included strange noises and the herding of unfamiliar groups of ostriches in nearby pens. They kept two groups of ostriches in lairage for one night. The one group was slaughtered first the next morning, whilst the other group of ostriches was kept until 16h00 that afternoon, adjacent to the stunning pen. The second group of ostriches thus had to endure a whole day of lairage stress (frequent movement of stockmen and other birds past their holding pen, unfamiliar noises commonly associated with abattoirs, etc.) before slaughter. The results of the meat analysis showed that the second group of ostriches that was subjected to lairage stress had higher muscle pH values after slaughter, as well as darker coloured muscles than the first group (Hoffman & Lambrechts, 2010). Both these characteristics are indicative of pre-slaughter stress and is an indication that lairage can contribute to *ante-mortem* stress.

2.8. CONCLUSION AND OBJECTIVES

As the export and local consumption of ostrich meat in South Africa and the world increases, it is becoming increasingly important that more emphasis is placed on producing higher quality ostrich meat in order to compete with other species such as cattle, sheep and poultry. One of the major determining factors in the quality of the ultimate meat product is the effect of pre-slaughter stress on animals. Relatively little information exists regarding this topic relating to ostriches and thus has very little been done to reduce the effects of stress on ostriches during this period. Relating this *ante-mortem* stress to the meat quality and blood haematology of ostriches is therefore essential to understanding the effect of *ante-mortem* stress and developing strategies to minimise its effects.

The objective of this study was to supply sound scientific research regarding the following aspects:
Comparing the effects of different transport distances on *ante-mortem* stress in ostriches as determined by blood haematology and *post-mortem* meat quality aspects.

Comparing the effects of different transport distances on the live weight loss encountered by ostriches during the *ante-mortem* period which has negative economical consequences for producers.
Determining the effect of farming system and the contribution of other factors such as road conditions, stocking density and floor type on the amount of bruises cut from ostrich carcasses during meat inspection at the abattoir.

Where applicable, correlations were verified between meat quality parameters and those factors which are known to influence them, such as ultimate pH_u. Correlations were also made between plasma levels of creatine kinase and drip loss to determine if creatine kinase, which is a measure of physical stress, also affected water-holding capacity of meat *post-mortem*.

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Chapter 3

EFFECT OF TRANSPORT-RELATED STRESS ON OSTRICH (*STRUTHIO CAMELUS*) HAEMATOLOGY AND CHEMISTRY VALUES

Abstract

Acute stress is known to affect blood haematological and blood chemistry values of animals. Few studies have comprehensively investigated this issue with specific regards to ostriches. The purpose of this study was to compare the effect of transport over varying distances on specific blood metabolites associated with stress in other animals, using an approach which would allow for quantification of the relative contributions of both psychological and metabolic stress. Sixteen ostriches were divided into two groups of eight each and transported for 60 and 600 km respectively, with blood samples obtained at various time points before and after transport. Both 60 and 600km distance transportation resulted in a significant increase in white blood cell (WBC) count, but increased heterophil:lymphocyte (H:L) ratio was only evident in the birds that travelled 600 km. Transport had no significant effect on corticosterone levels. Both transport distance and time significantly increased concentrations of indicators of physical stress, such as s-AST and s-CK, although appearance in circulation occurred at different points in time after transport. These increases most likely indicate severe physical stress, ascribed to the effort required for maintenance of posture and balance on a moving vehicle. In conclusion, this study indicates that physical stress associated with transport during the *ante-mortem* period leads to muscle damage, which adversely affects meat quality, and that psychological stress is not induced by transport itself, but rather by acute incidents before and after transport, during the *ante-mortem* period.

INTRODUCTION

The South African Black ostrich (*Struthio camelus* var. *domesticus*) is the most commonly found ostrich species in South Africa (Swart, 1988). The ostrich belongs to the family of ratites, a group of flightless birds that also includes the emu, cassowary, rhea and the kiwi (Van Tuinen *et al*., 1998). Ostrich farming has only become an alternative form of agriculture over the past four decades (1970-2010) as a means to produce ostrich meat as one of the primary products (McKeegan & Deeming, 1997). Consumers of meat have particularly become more aware of the nutritional quality and the products' effects on human health (Hoffman *et al*., 2005). The fact that ostrich meat has higher levels of polyunsaturated fatty acids (PUFA) (Horbanczuk *et al*., 1998; Girolami *et al*., 2003) means that consumers classify ostrich meat as a healthy meat that may minimise illnesses such as coronary heart disease (Sales & Oliver-Lyons, 1996; Balog & Almeida Paz, 2007). As a result of the development of the ostrich industry, the number of ostriches being slaughtered increased drastically.

According to Wotten and Hewitt (1999), the transportation of ostriches to the abattoir is considered to be the most stressful form of management during the life of these birds. Stress during the *ante-mortem* period leads to a decrease in the quality of the carcass, as well as the incidence of mortalities (Wotten & Hewitt, 1999). The quantitative assessment of stress has not been adequately related to animal welfare and facts around

this subject are still an ongoing debate (Kamau *et al*., 2002). Moberg (1985) proposed that biological responses to stress may be valuable to assess animal wellbeing. Knowledge of the general and stress-related blood biochemistry and haematology of the ostrich is thus necessary to monitor the health of ostriches and correctly diagnose and treat diseases (Bouda *et al*., 2009).

There is very little literature available regarding the transportation of slaughter ostriches and the effect transportation has on blood biochemical and haematological parameters. Only a few papers have reported on the blood metabolites of adult ostriches under normal circumstances (baseline) (Van Heerden *et al*., 1985; Levy *et al*., 1989; Okotie-Eboh *et al*., 1992; Mushi *et al*., 1999; Verstappen *et al*., 2002). The only other studies that could be sourced that reported on blood metabolite concentrations before and after transport, were that of Mitchell *et al*. (1996) and Minka and Ayo (2008).

As there has been so little research on the effect of transport systems on the welfare of ostriches (Wotten & Hewitt, 1999), no improvements have been made to these transport systems to improve the wellbeing of slaughter ostriches. To address and minimise the problem of transport stress, the magnitude and types of stress encountered over varying distances by using specific blood metabolite indicators commonly used to determine stress in other birds, such as corticosterone, heterophil:lymphocyte (H:L) ratio, creatine kinase and aspartate aminotransferase were measured.

Therefore, the aim of this study was to determine the effect of *ante-mortem* stress in response to different transport distances/periods on the blood metabolites of slaughter ostriches. Specific objectives of this study were to:

Assess transport-related stress in ostriches.

Distinguish between psychological and metabolic stress assessment of the corticosterone response biochemical parameters respectively.

Investigate the ability of transported birds to recover from transport-related stress overnight, prior to slaughter.

MATERIALS AND METHODS

Experimental Animals

For this study, 16 South African Black ostriches (*Struthio camelus*), of approximately 10 months of age from the Kromme Rhee experimental farm outside of Stellenbosch, were used. All 16 ostriches received the same commercial diet before slaughter and were raised under feedlot conditions. The 16 birds were divided into two groups of eight, and each group consisted of four male and four female ostriches. The trial was conducted from 5 to 6 May 2009. Ethical approval for the study was obtained from the Stellenbosch University Sub-committee B Animal Research Ethics committee (reference number, 2009B03003).

Experimental design

The experimental design of this trial is a completely randomised design with three treatments (transport distance) and eight birds per treatment. Ethical approval for the study was obtained from the Stellenbosch University Sub-committee B Animal Research Ethics committee (reference number, 2009B03003).

Transport and Slaughter

Treatments B and C were the two groups of ostriches that travelled 600 and 60 km respectively to the abattoir. The ostriches used in the trial were familiar with humans and handling by humans due to the fact that they were raised in a feedlot and were in contact with people on a daily basis.

The birds travelled in a specially designed trailor for the transportation of ostriches. The trailer consisted out of two compartments whilst there were two female and two male birds in every compartment with an average floor space of 0.5 m^2 per bird. The trailor was pulled by a 4×4 vehicle whilst the trailor's floor had steel grids. During transport of the birds in treatment B the driver, who had experience in transporting ostriches, was only allowed to stop twice to refuel the vehicle. The driver was not allowed to stop when he transported the birds in treatment C. At all times the driver had to keep a maximum speed of 100 km/h.

At the abattoir birds were handled by experience handlers and placed into lairage pens adjacent to the abattoir. Birds had access to water whilst in lairage whilst the floor of the lairage pen was concrete. Both these groups were slaughtered at Swartland commercial abattoir in Malmesbury, the day after they were transported.

Collection of Blood Samples

Blood samples (2 tubes, SST and EDTA at each time) were collected from the jugular vein (every time) of each ostrich in the two groups of birds that were transported for 600 (treatment B) and 60 km (treatment C) respectively. Each time blood was collected, birds were placed in a crush and a sock placed over their head to blind them. This was done to calm the ostrich during blood sampling and weighing. The first samples were taken at 07h00 (baseline) after which the birds of treatment B were loaded onto the trailer and transported to Malmesbury. After the ostriches of treatment B arrived at the abattoir (13h00), from a 600 km trip, another sample was taken from each bird in group B. The birds of treatment C remained on the farm whilst the birds of treatment B were being transported. At 15h30, the same trailer went back to the farm to collect the birds of treatment C. Whilst being loaded onto the trailer an additional pre-transport blood sample were taken from the birds in treatment C after which they were subjected to a 60 km journey. Blood samples were again obtained from each of the ostriches in the treatment C group upon their arrival at the abattoir at 16h30 (post-transport). Blood samples were also collected from each of the three groups of ostriches (treatment B and C) after an overnight recovery period, before and after stunning and exsanguination.

For all samples, one tube (SST) each were then kept on ice and the other (EDTA) at room temperature for approximately two to three hours, during which time the samples were transported to the Department

Physiological Science of the University of Stellenbosch. Table 3.1 gives a summary of the times and type of blood samples obtained from each of the two treatments during the trial.

Table 3.1
Summary of the times and types of blood samples obtained from treatment B and C at different stages during the trial

Time of Blood Sample	Time	Blood Sample Type	
		Transport Groups	
		B (600 km)	C (60 km)
Baseline	07h00	SST & EDTA	SST & EDTA
Post-Transport	13h00	SST & EDTA	
Pre-transport	15h30		SST & EDTA
Post-transport	16h30		SST & EDTA
Prior to slaughter	08h00 (Following day)	SST & EDTA	SST & EDTA

Blood analysis

The SST-clotted samples were taken to the Department Physiological Sciences at the University of Stellenbosch where the samples were centrifuged at 4 ºC for ten minutes at 3000 rpm, serum aliquoted into 2 clean tubes per sample and frozen at -80 ºC within 4 hours after collection. All samples were batched to be analysed on the same day, to eliminate day-to-day variation of analytical instruments. One of the frozen serum samples of each of the SST-clotted samples was then sent to the veterinary pathology laboratories (Vetlab) in Parow for determination of the concentrations of serum protein, albumin, globulin aspartate aminotransferase (s-AST) and creatine kinase (s-CK). The other serum aliquot was used to determine the serum corticosterone levels (ng/ml), using a rodent EIA kit (AC-14F1, Octeia, Immunodiagnostics Systems) at the Department Physiological Sciences of the University of Stellenbosch. The other sample tubes (EDTA) were analyzed within four hours of collection to determine the full blood and differential white blood cell counts of the ostriches using automated analysis on a haematological cytometer (Celldyne 3700CS with VetPack software, Abbott Diagnostics).

Statistical analysis

Data was analysed using Statistica v.8. Analyses and included ANOVA with the Fisher LSD (least significant difference) test as the *post hoc* test. In order to include a random variable, and to take the slightly unbalanced nature of the data into account, the data was analysed by means of mixed models with the animal ID, nested in group, as a random variable. Since each group consisted of ostriches of the same age (10 months) and contained equal numbers of female and male birds, the only main effects considered, when analysing the data, were the treatment effect and the effect time had (pre-transport vs post-transport etc.) on the blood haematology values. The differences between transport distance (i.e. treatments) and times (within treatments) were, where appropriate, tested by means of the null hypothesis (H_o), with H_o: $\mu_1 = \mu_2$ and the alternate hypothesis (H_a) being that H_a: $\mu_1 \neq \mu_2$. Data are presented as means and standard errors and the Fisher LSD test was used to determine whether there were differences in the means of the groups involved in the study. A p-value of < 0.05 was accepted as statistically significant**.**

RESULTS AND DISCUSSION

Total Protein, Albumin and Globulin concentrations

In the current study there was no significant difference between total protein, albumin and globulin plasma concentrations of the two groups of ostriches at any time point (Table 3.4). This was also the case for the total protein, albumin and globulin concentrations within each group at baseline and prior to slaughter, indicating that protein catabolism and dehydration didn't have a significant effect on total protein levels in the current trial. According to Warriss *et al*. (1995) and Knowles *et al*. (1999) when animals are transported for long periods, there is an increase in the plasma levels of total protein, globulin and albumin, partly due to dehydration and the immobilisation of body reserves through protein catabolism.

Total white blood cell counts and Heterophil:Lymphocyte ratio

Table 3.2 illustrates the mean white blood cell counts at each stage for the two groups of ostriches that were transported. There was no difference in the average white blood cell counts at baseline (P = 0.606) between the two groups of ostriches that were transported for 60 and 600 km respectively. Palomeque *et al*. (1991) reported a slightly lower WBC count for adult Masai ostriches ($21.0 \pm 8.0 \times 10^3$ cells/μL). Mushi *et al*. (1999) and Minka and Ayo (2008) reported even lower WBC counts of $5.0 \pm 1.8 \times 10^3$ cells/μL and $2.0 \pm 0.2 \times 10^3$ cells/μL respectively for adult ostriches. However, these differences are probably species-specific, and not directly indicative of differences in immune function.

After transport there was a significant difference between the WBC of groups B and C (P = 0.035). Group C showed no increase in their WBC count after transport (P = 0.565) whilst group B had a significant increase (P = 0.002) in their WBC count post-transport. Minka and Ayo (2008) found no difference in the leukocyte count before ($2.0 \pm 0.2 \times 10^3$ cells/μL) and after transport ($2.4 \pm 0.8 \times 10^3$ cells/μL). Prior to slaughter, both groups had increased WBC counts that did not differ between treatments (P = 0.805). The ostriches of

treatment B's WBC count remained stable compared to the level after transport (P = 0.996) during lairage. This meant that the ostriches that travelled 60 km had a significant increase in their WBC count during lairage (P = 0.003) compared to the WBC count taken of the same group just after transport. However, overall there were significant increases in both treatments B and C's white blood cell counts during the whole *ante-mortem* period. This could mean that a journey of 60 km could not manifest an immediate change in the WBC count after transport, but that the WBC changes took place during lairage. Changes that occur in the WBC count during periods of stress may be time-dependant i.e. it takes a certain period of time after a stressful incident to manifest changes in WBC count in the plasma of ostriches. This explains why the WBC changes in the ostriches of treatment C were only seen the morning after the birds were transported. Nevertheless, this result suggests that during transport, cell damage occurred that lead to an inflammatory response and thus an increase in the amount of white blood cells into the blood from the spleen and bone marrow, which was measured at different times during the *ante-mortem* period.

Table 3.2

Mean White Blood Cell counts as measured at four different time intervals during the transportation and lairage of the 2 groups of ostriches that were transported to the abattoir.

Time of Blood Sample	White Blood Cell Count (Mean ± s.e.) (× 103 cells/mm^3)		Comparison of Transport groups P < |t|
	Transport Groups		
	B (600 km)	C (60 km)	
Baseline at 07h00	28.74^{b} ± 3.065	26.49^{b} ± 3.065	0.607
After Transport	37.51^{a} ± 3.065	28.05^{b} ± 3.065	0.035
Prior to Slaughter	37.5^{a} ± 3.065	36.42^{a} ± 3.065	0.805
After Slaughter	37.28^{a} ± 3.743	34.53^{a} ± 3.065	0.702

$^{a-c}$ Means in columns ,within a group, with different superscripts are significantly different, P < 0.05.

Since total WBC may be influenced by numerous factors, and as it is made up of different cells with varying functions, this measurement is too crude to draw a conclusion in the context of the current study. It is therefore necessary to consider more refined measures. According to Gross and Siegel (1983) the H:L ratio in blood is a very good indicator of stress in avian species. They proposed that the H:L ratio would be a more accurate measure of long-term changes in the environment and that the H:L ratio was poorly correlated to the glucocorticoid corticosterone, which was in turn a better indicator of short-term changes in the environment of an animal (Gross & Siegel, 1983).

There were no differences (P = 0.240) in the H:L ratio at baseline between the treatment groups B and C. The baseline H:L ratios in the current study are in the same range as reported by Minka and Ayo (2008) and

Spinu *et al*. (1999) for adult ostriches, when they reported H:L ratios of 2.12 ± 0.1 and 2.72 ± 0.84 respectively. Mitchell *et al*. (1996) reported a much larger H:L ratio (8.5 ± 3.2) at baseline before transport than the current study. The only difference in the H:L ratio of the two groups was measured after transport. Post-transport the birds of treatment B had a higher H:L ratio than their baseline ratio and also had a significantly higher H:L ratio than the birds of treatment C, whose H:L ratio remained constant throughout the whole trial. Mitchell *et al*. (1996) reported an increase from 8.5 ± 3.2 to 24.2 ± 10.3 due to transport. Although the H:L ratio increase in the current study was not as profound as the increase reported by Mitchell *et al*. (1996), there seems to be a tendency for the H:L ratio to increase if birds are exposed to periods of stress. The amount of heterophils increases after transport in broilers whilst the amount of lymphocytes decreases at the same time, ultimately leading to an increased H:L ratio in the plasma of broilers (Mitchell *et al*., 1992).

Table 3.3

Mean Heterophil: Lymphocyte ratio as measured at four different time intervals during the transportation, lairage and slaughter of the two groups of ostriches that were transported to the abattoir.

Time of Blood Sample	Heterophil:Lymphocyte Ratio (Mean ± s.e.)		Comparison of Transport groups $P < \|t\|$
	Transport Groups		
	B (600 km)	C (60 km)	
Baseline at 07h00	1.73^{b} ± 0.374	1.10^{a} ± 0.374	0.240
After Transport	2.72^{a} ± 0.374	0.86^{a} ± 0.374	0.001
Prior to Slaughter	1.19^{b} ± 0.374	0.90^{a} ± 0.374	0.590
After Slaughter	0.67^{b} ± 0.513	0.87^{a} ± 0.374	0.754

$^{a-c}$ Means in columns, within a group, with different superscripts are significantly different, P < 0.05.

The changes in the individual heterophil and lymphocyte counts may also be due to other factors such as exposure of the animal/bird to novel pathogens, and dehydration of the animal during periods such as transport during which the animal is normally deprived of water, as was the case in the current study. The individual heterophil percentages of groups C and B respectively did not increase significantly over the *ante-mortem* period, from pre-transport to slaughter (Table 3.4). According to the literature, heterophil counts increase in periods of stress, e.g. Minka and Ayo (2008) found increases in the heterophil percentage from pre-transport (60.1 ± 2.0 %) to post-transport (78.4 ± 2.4 %). This increase in heterophil count is probably due to cell damage that occured during transport and causes inflammation.

The individual lymphocyte percentages of both groups of ostriches remained constant during each phase of the trial, with both groups not differing from each other at any of time points (Table 3.4) in the current study. Minka and Ayo (2008) reported a decrease in the percentage lymphocytes from pre-transport (28.4 ± 2.7 %)

to post-transport (19.7 ± 1.7 %). This meant that even though there were no significant changes in the heterophil and lymphocyte percentages during the *ante-mortem* period of both groups (treatment C and B), the combined subtle changes in the individual heterophil and lymphocyte values led to a significant increase post-transport in the H:L ratio (Table 3.3) of the birds that travelled 600 km.

Table 3.4 gives the mean cell counts of all five leukocytes. As mentioned, the heterophil count increases and the lymphocyte count decreases in avians when they are subjected to chronic stress (Gross & Siegel, 1983; Minka & Ayo, 2008). However, there is still no clarity on the effect either chronic or acute stress has on the other three leukocytes measured. According to Ayo *et al*. (2005) the eosinophil count decreases after transportation, but this was not the case in transported ostriches in the study by Minka and Ayo (2008) who reported no effect of transport.

In the current study there was a significant increase in the eosinophil percentage after transport ($P < 0.01$) in the ostriches of treatment B and the eosinophil levels of the same group remained elevated after lairage (prior to slaughter). Similar to the total WBC count, the increases ($P < 0.01$) in the eosinophil count only manifested after lairage in the birds of treatment C. However, given the limited literature on this topic, and the fact that eosinophils are actually associated with allergic reactions and defence against parasites (Weller, 1997), it is not possible to draw a conclusion regarding this result.

In the current study there was no difference in the monocyte counts between the two groups at any stage, as well as no difference within groups at any stages during the trial. Mushi *et al*. (1999) reported that the average relative monocyte counts for adult ostriches were 1 ± 0.5 %, whilst Minka and Ayo (2008) found a similar value to that in this investigation. Minka and Ayo (2008) also reported no differences in the monocyte count of ostriches before and after slaughter and measured monocyte counts of 2.2 ± 0.7 and 2.8 ± 1.2 % respectively. The results in the current study correspond to the values reported by Minka and Ayo (2008), suggesting no effect of acute transport-related stress on this cell count.

The basophil count had a tendency to decrease during lairage in the current study. There were significant differences ($P = 0.024$) in basophil counts between the groups that travelled 60 km and 600 km respectively at baseline (before transport). The reason for this is unknown, but given the small number of cells, this difference is unlikely to have clinical significance. According to Mushi *et al*. (1999), the relative basophil count for adult ostriches was 6 ± 1.4 %. This is much higher than the 0.2 ± 0.3 % reported by Minka and Ayo (2008). Spinu *et al*. (1999) reported intermediate values for the basophil count of South African Black and Red Neck adult ostriches (3.29 ± 1.93 and 1.82 ± 1.02 %, respectively). After transport, treatment B had a significant decrease ($P < 0.01$) in basophil percentages, whilst there were no differences ($P = 0.28$) in the basophil counts, before and after transport, in the birds of treatment C. The birds of treatment C ultimately had a significant decrease ($P = 0.018$) in basophil counts from pre-transport to just prior to slaughter. The ostriches of treatment B also had a significant decrease in basophil percentages from baseline to just prior to slaughter ($P < 0.01$). Minka and Ayo (2008) reported a basophil percentage of 0.4 ± 0.2 % after transport of

ostriches and this value was similar to the basophil percentage pre-transport. The basophil percentages in the current study lay intermediate to that of Spinu *et al*. (1999) and Minka and Ayo (2008). In both treatments B and C in the current study, the basophil percentages decreased during the *ante-mortem* period (transport and lairage) of ostriches. According to Mitchell *et al*. (1996) transport can cause tissue damage. When there is damage to muscle tissue, mast cells derived from circulating basophils play a key role in the repair of the damaged tissue. The migration of inflammatory cells such as basophils/mast cells to areas of tissue damage may play a role in the decreased amount of circulating basophils (Martin & Leibovich, 2005).

Table 3.4

The mean blood haematology counts as measured at three different time intervals during the transportation and lairage of the two groups of ostriches that were transported.

Blood Parameter	Time of Blood Sample		Comparison of Transport Groups	Time of Blood Sample		Comparison of Transport Groups	Time of Blood Sample		Comparison of Transport Groups
	Baseline (Means ± s.e.)			After Transport (Means ± s.e.)			Prior to Slaughter (Means ± s.e.)		
	Transport Groups		P < |t|	Transport Groups		P < |t|	Transport Groups		P < |t|
	B (600 km)	C (60 km)		B (600 km)	C (60 km)		B (600 km)	C (60 km)	
Total Protein (g/L)	41.21^{a} ± 2.552	37.63^{a} ± 2.466	0.319	40.13^{a} ± 2.466	37.63^{a} ± 2.466	0.478	43.50^{a} ± 2.466	40.00^{a} ± 2.466	0.322
Albumin (g/L)	22.35^{ab} ± 0.965	20.63^{a} ± 0.941	0.208	21.75^{b} ± 0.941	20.75^{a} ± 0.941	0.457	23.25^{a} ± 0.941	21.75^{a} ± 0.941	0.267
Globulin (g/L)	18.90^{a} ± 1.779	17.00^{a} ± 1.706	0.445	18.38^{a} ± 1.706	16.88^{a} ± 1.706	0.538	20.25^{a} ± 1.706	18.25^{a} ± 1.706	0.412
Basophils (%)	1.98^{a} ± 0.258	1.16^{a} ± 0.229	0.024	0.76^{b} ± 0.229	1.48^{a} ± 0.258	0.046	0.75^{b} ± 0.242	0.50^{b} ± 0.229	0.464
Monocytes (%)	2.18^{a} ± 0.362	1.41^{a} ± 0.362	0.137	1.65^{a} ± 0.362	2.15^{a} ± 0.362	0.340	1.33^{a} ± 0.362	1.23^{a} ± 0.362	0.853
Eosinophils (%)	5.24^{c} ± 1.177	4.87^{b} ± 1.139	0.819	12.2^{a} ± 1.139	6.20^{b} ± 1.139	0.001	9.35^{b} ± 1.139	10.33^{a} ± 1.139	0.550
Heterophils (%)	40.5^{a} ± 3.799	35.83^{a} ± 3.799	0.390	43.71^{a} ± 3.799	31.94^{a} ± 3.799	0.035	37.78^{a} ± 3.799	31.61^{a} ± 3.799	0.259
Lymphocytes (%)	38.85^{a} ± 7.419	42.76^{a} ± 7.419	0.711	22.40^{a} ± 7.419	42.7^{a} ± 7.419	0.060	34.78^{a} ± 7.419	38.4^{a} ± 7.419	0.732

$^{a-c}$ Means in rows, within a group, with different superscripts are significantly different, P < 0.05.

Corticosterone

According to Kamau *et al*. (2002) the pathophysiological response to stress in avian species is mediated by an increase in the corticosterone concentration in the plasma. Levels of glucocorticoids (of which corticosterone is one) are commonly used to determine the levels of stress in animals (e.g. Wingfield, 1994).

A summary of all corticosterone results obtained in the current study is presented in Table 3.5. There were no differences (P = 0.434) in the baseline corticosterone concentrations of groups C and B respectively (Figure 3.1). The corticosterone concentration at baseline in the current study was higher than the baseline corticosterone concentration in Greater Rheas (3.98 ± 1.04 ng/ml) (Leche *et al.,* 2009). The baseline corticosterone values reported by Leche *et al.* (2009) for Greater Rheas were similar to the baseline values reported by Mitchell *et al*. (1996) in ostriches (4.9 ± 2.9 ng/ml). In the study by Mitchell *et al*. (1996) 50 ten-month old ostriches were transported for a 4.5 hour period as a stress intervention. They took baseline (before transport) and after transport blood samples of 8 of the 50 ostriches in the trial.It is unknown why the birds in the current study had higher baseline corticosterone levels than the levels reported by Mitchell *et al*. (1996) and Leche *et al*. (2009) for ostriches and Greater Rheas respectively. In the case of the Greater Rheas, 10-month old birds were used in the trial and blood was collected from the jugular vein of each bird between 07h00 and 08h00. These Greater Rheas were also raised in captivity and had access to feed throughout the trial. All of these factors mentioned in the trial by Leche *et al*. (2009) were similar to the procedure in the current study. Thus, it cannot be discarded that a species difference could account for the differences seen in the corticosterone concentration in the current study and the study by Leche *et al*. (2009). No information could be sourced on issues such as fasting, time of blood sample collection, how birds were raised and which area on the body of the birds were used to obtain blood samples (for instance the neck or in the wing) in the study by Mitchell *et al*. (1996). For example, if birds in the trial by Mitchell *et al*. (1996) had their baseline blood samples taken at later stages in the day (other than between 07h00 and 08h00), circadian rhythm could well account for the fact that the corticosterone baseline corticosterone differed from the concentration in the current study. The differences seen in the baseline corticosterone concentration of Mitchell *et al*. (1996) and the current study could also have been due to differences in the handling and constraint of the birds in each study before blood samples were taken. If one of the studies' handling methods inflicted more pain and stress prior to blood sampling, it could well have influenced the baseline corticosterone concentrations of the birds.

Table 3.5

Mean Corticosterone concentration as measured at 4 different time intervals during the transportation, lairage and slaughter of the two groups of ostriches that were transported to the abattoir.

Time of Blood Sample	Corticosterone Concentration (ng/ml) (Mean ± s.e.)		Comparison of Transport Groups P < |t|
	Transport Groups		
	B (600 km)	C (60 km)	
Baseline at 07h00	16.71[a] ± 2.932	13.43[c] ± 2.932	0.434
After Transport	11.98[a] ± 3.123	27.57[b] ± 2.932	0.001
Prior to Slaughter	10.28[a] ± 3.123	25.78[b] ± 2.932	0.001
After Slaughter	11.74[a] ± 3.123	37.03[a] ± 2.932	0.000

[a-c] Means in columns, within a group, with different superscripts are significantly different, P < 0.05.

The post-transport corticosterone concentration of the ostriches in treatment C was higher (P = 0.001) than the corticosterone concentration of the ostriches in treatment B. The corticosterone concentration in this group (treatment C) remained significantly higher for the duration of the protocol, both in comparison to baseline and Group B (Figure 3.1). This is a somewhat unexpected finding, since group B was exposed to a longer duration of transport. However, there could be more than one possible explanation for this finding.

The group of ostriches that travelled only 60 km (Treatment C) remained in their paddocks without feed for several hours until 15h30 when a second blood sample was taken prior to transport (Figure 3.1). This blood sample indicated the highest mean corticosterone concentration (45.24 ± 4.02 ng/ml) throughout the duration of the trial and was significantly higher (P = 0.001) than the corticosterone concentration after transport of the same group (60 km group) (Figure 3.1). This major increase in the corticosterone concentration in this group probably indicates that these birds suffered from an acute stress exposure prior to transport that was not directly related to transport. The response seen may have been in reaction to the deprivation of feed, resulting in an increase in corticosterone concentration for maintenance of metabolic homeostasis. However, food deprivation is unlikely to be a major stressor, since serum protein and albumin levels did not differ (Table 3.4), which suggests that no protein catabolism was initiated, as would be expected in starvation conditions. More likely, this particular group was exposed to an acute stressful episode during this waiting period in their familiar paddocks and surroundings, given the fact that the baseline corticosterone levels for all groups were higher than those reported by other authors, which supports the interpretation that a stressful environment may account for the result seen. Unfortunately, at this time a definite conclusion cannot be drawn, since insufficient proof for either option is available.

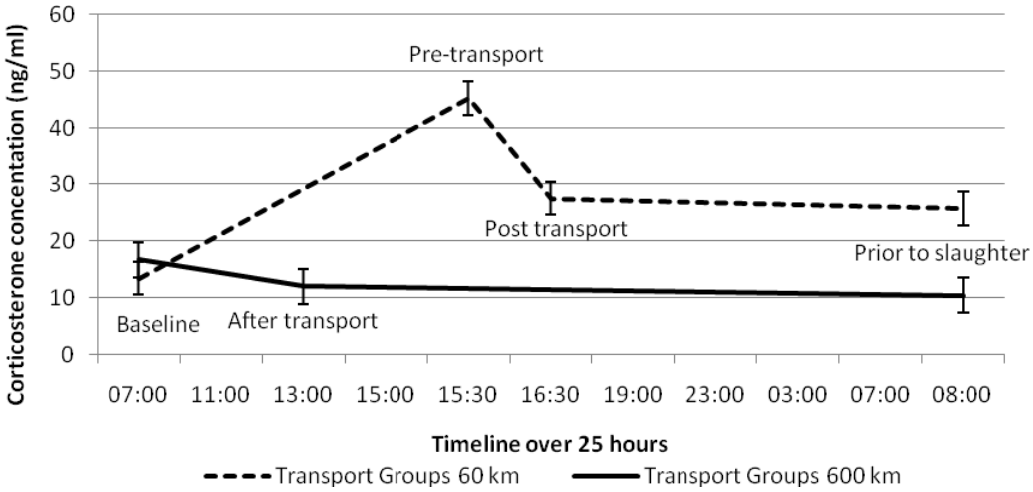

Figure 3.1 Mean corticosterone concentration over a period of 25 hours for ostriches that travelled 60 km (n=8) and 600 km (n=8) respectively to the abattoir

When Leche *et al*. (2009) injected Greater Rheas with ACTH, they found that the corticosterone concentration increased from 3.98 ± 1.04 to 89.83 ± 12.42 ng/mL within 15 minutes. The magnitude of the response is much greater than the reports of the current study, but it is not always possible to place the results of field studies into context to experimental conditions, due to the non-physiological nature of some of these protocols. In a study more similar to the current study, Mitchell *et al*. (1996) observed a 2-fold increase in plasma corticosterone concentration of 10-month old ostriches after they were transported for 4.5 hours. Although birds were of similar age, other confounding factors such as nutrition and transport conditions may have played a big role in the magnitude of the stress response seen in the current study and the study by Mitchell *et al.* (1996). In the current study, there was a 3-fold increase in plasma corticosterone levels, of the birds that travelled 60 km, during the feed deprivation and pre-transport period which is similar to the magnitude increase observed by Mitchell *et al*. (1996).

When interpreting data obtained in field conditions, several confounding factors have to be considered. For example, another factor that can also have an influence on the corticosterone concentration during the different periods of the day is the fact that avian species have a diurnal rhythm of corticosterone that fluctuates during the day. The first peak is just before dawn whist the second peak is in the afternoon (Van Cauter, 1989). Humans are also non-nocturnal organisms and exhibit diurnal variations in their plasma corticosteroid (cortisol) concentrations over a 24-hour period (Van Cauter, 1989). According to Chan and Phillips (1973), ducks (*Anas platyrhynchos*) have two similar peaks in plasma corticosterone concentration over a 24-hour period. The first peak is in the period before dawn after which the corticosterone concentration drops steadily. The second peak is then reached in the late afternoon around 16h00 (Chan & Phillips, 1973). No literature could be sourced that reported on diurnal rhythms in ratites. As only the

baseline and the pre-slaughter corticosterone concentration were taken at the same time point for both of the two transported groups of ostriches and thus the possibility that the diurnal rhythm of corticosterone in plasma has an effect on the values of corticosterone at the 13h00, 15h30 and 16h30 intervals (although the levels observed at 15h30 and 16h30 for the 60 km group were above the normal range for these animals) cannot be ruled out. The fact that the corticosterone concentration of the ostriches in treatment C remained significantly elevated after lairage (before slaughter), compared to the ostriches of treatment B, indicates that these birds (60 km group) were more stressed and that the stress had a longer lasting effect on these birds (Figure 3.1). The fact that the birds of treatment C had their highest corticosterone concentration prior to transport, suggests the high corticosterone concentrations of the birds in treatment C cannot be related to transport induced stress. While treatment B had recovered in terms of corticosterone levels after overnight lairage, this recovery period does not seem to have been sufficient for the group of ostriches that travelled 60 km, suggesting that a truly stressed bird will need longer recovery prior to slaughter than is currently common practise.

Creatine Kinase and Aspartate Aminotransferase

Creatine Kinase and AST are indicators of muscle damage in skeletal and cardiac muscles (Quintavalla *et al*., 2001) and the increased permeability of the muscle membrane when it is damaged leads to these enzymes leaking into the plasma (Warriss *et al*., 1995; Tadich *et al*., 2005).

There was no difference (P = 0.882) in the creatine kinase baseline concentrations of groups C and B respectively (Table 3.6). Van Heerden *et al*. (1985) reported that the range for CK in ostriches lies between 394-2500 IU/L at baseline without subjecting the birds to physical stress. Other authors such as Levy *et al*. (1989) and Palomeque *et al*. (1991) also reported baseline concentrations of CK that fit into the range reported by Van Heerden *et al*. (1985). Baseline values reported in the current study were slightly higher, but comparable to these reported values. However, after transportation, the group of ostriches that travelled the furthest (600 km) exhibited the most significant increase in plasma creatine kinase concentration (P = 0.004) from baseline to after transport (Figure 3.2) indicating that the birds of treatment B probably suffered the greatest muscle damage during transport.

Table 3.6

The influence of transport distance, and lairage duration on the mean creatine kinase concentration measured at four different time intervals in slaughter ostriches transported over 60km and 600km to an abattoir, respectively.

Time of Blood Sample	Creatine Kinase (IU/L) (Mean ± s.e.)		Comparison of Transport groups P < |t|
	Transport Groups		
	B (600 km)	C (60 km)	
Baseline at 07h00	2710.37[c] ± 2528.743	3232.13[b] ± 2392.988	0.882
After Transport	11714.38[b] ± 2392.988	3935.63[b] ± 2392.988	0.027
Prior to Slaughter	16035.38[ab] ± 2392.988	14798.75[a] ± 2392.988	0.717
After Slaughter	19879.76[a] ± 2528.743	18335.01[a] ± 2528.641	0.668

[a-c] Means in columns, within a group, with different superscripts are significantly different, P < 0.05.

As Figure 3.2 indicates, there is a pronounced increase (P < 0.01) in the CK concentrations of the birds in treatment C, but only after the lairage period, whereas the birds in treatment B had a similarly elevated CK concentration (P = 0.13) after transport which did not recover during lairage. Kannan *et al*. (2000) reported that there was a lag time from the period of physical stress (transportation) to the time when an increased CK concentration was seen in the plasma of goats. This can be ascribed to the fact that these enzymes have to be cleared from injured tissues into the blood before detection is possible. This may be the reason why an increased CK concentration was not observed in the blood of the birds in treatment C. Just prior to slaughter, both groups of birds had similar CK concentrations (P = 0.717) (Table 3.6). In the only other study that reported on transport-related changes in CK concentrations, Mitchell *et al*. (1996) reported values of 560 ± 53 and 1398 ± 104 IU/L, before and after transport respectively. In this study the birds travelled between 4 and 5 hours (Mitchell *et al*., 1996). The baseline and after transport CK values of Mitchell *et al*. (1996) were considerably less than the CK values reported in the current study. This may have been due to certain factors during transport (e.g. birds sitting some of the time). Increased stocking density, and the fact that animals have to remain standing during transport, also lead to increased levels of plasma CK that is indicative of physical fatigue according to Knowles *et al*. (1999). However, the same trend emerged in both studies indicating that plasma CK concentration increases during transport of ostriches.

Transportation is physically demanding. Birds have to maintain their balance at varying speeds and during direction changes for the duration of transport and are also subjected to bruising during this period. Both these factors affect the permeability of the muscle membranes and the amount of enzymes released into the plasma (Brown *et al*., 1999a). The fact that the birds of treatment B and C still had elevated CK levels after about 18 hours, confirms the results reported by Knowles *et al*. (1999) that plasma CK levels remain

elevated for long periods of time. According to Knowles and Warriss (2000), transport of animals can still have an effect on plasma CK levels up to a few days after transport and can take up to a week to recover to normal levels.

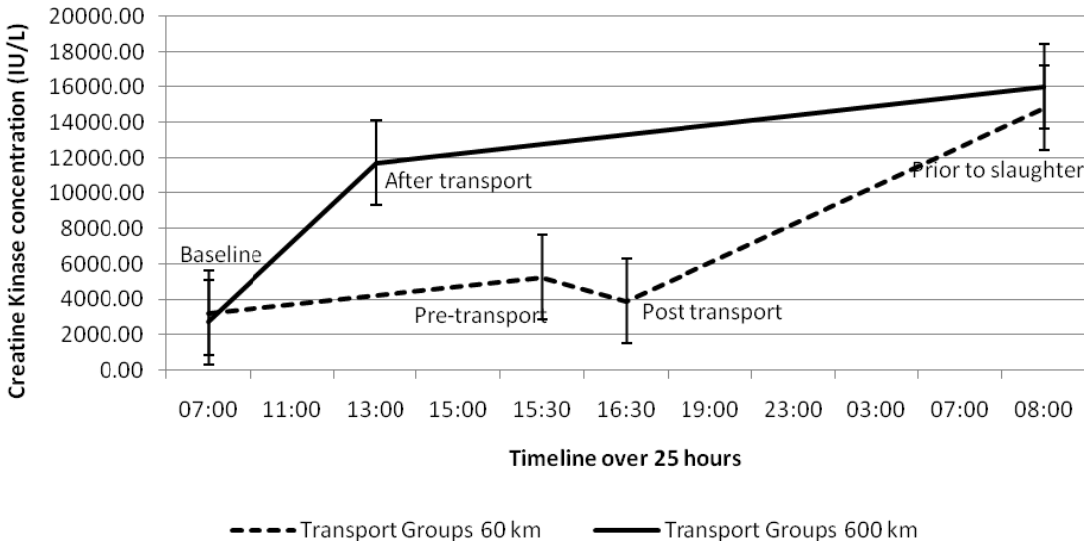

Figure 3.2 Mean creatine kinase concentration over a period of 25 hours for ostriches that travelled 60 km (n=8) and 600 km (n=8) respectively to an abattoir.

Plasma aspartate aminotransferase baseline concentrations did not differ (P=0.55) between samples obtained from Group B and C. Van Heerden *et al*. (1985) reported that the range for AST lies between 100-892 IU/L for ostriches at baseline, i.e. for birds that were not subjected to any extent/degree of extreme physical stress such as transportation. Other authors such as Levy *et al*. (1989) and Palomeque *et al*. (1991) also reported baseline concentrations of AST that, similar to this investigation, fit into the range reported by Van Heerden *et al*. (1985).

After transportation the ostriches of treatment B showed a significant increase in plasma AST concentration (P = 0.032) from pre-transport to after transport (Figure 3.3). The ostriches in treatment C only had a significant increase in AST levels after lairage. It seems that similar to CK, the enzyme AST takes time to manifest changes in the plasma concentration after a period of physical stress. Prior to slaughter there was no difference in the plasma AST concentration between the two groups (Table 3.7). Mitchell *et al*. (1996) also reported pre-transport and post-transport AST concentrations of 115 ± 6.7 IU/L and 137 ± 3.6 IU/L respectively. The AST values at baseline and after transport in both of the groups in the current trial were substantially higher than the results reported by Mitchell *et al*. (1996). However, both the current study and the study by Mitchell *et al*. (1996) observed significant increases in plasma AST concentration as a result of transport.

Table 3.7

Mean aspartate aminotransferase concentration as measured at four different time intervals during the transportation and lairage of the two groups of ostriches that were transported different distances to the abattoir.

Time of Blood Sample	Aspartate Aminotransferase (IU/L) (mean ± s.e.)		Comparison of Transport Groups $P < \|t\|$
	Transport Groups		
	B (600 km)	C (60 km)	
Baseline at 07h00	224.90^{c} ± 32.602	252.0^{b} ± 30.565	0.548
After Transport	318.63^{b} ± 30.565	279.5^{b} ± 30.565	0.371
Prior to Slaughter	336.88^{b} ± 30.565	412.63^{a} ± 30.565	0.088
After Slaughter	462.13^{a} ± 32.602	465.53^{a} ± 32.602	0.942

$^{a-c}$ Means in columns, within a group, with different superscripts are significantly different ($P < 0.05$)

It was expected that the birds that travelled the furthest (600 km) would exhibit the highest plasma concentration of AST due to the fact that AST is an indication of the amount of physical stress and muscle damage endured during travel. It was postulated that the birds that travelled 600 km would be more prone to fatigue, muscle bruising and exhaustion because they had to maintain balance and postural stability for far greater periods. These factors all affect permeability of enzymes such as AST through the muscle membrane (Brown *et al*., 1999a). It is evident in the current study that plasma AST concentration, like plasma CK, is also influenced by lag time. This observation was made due to the fact that AST levels increased during transport of the ostriches that travelled 600 km whilst the birds that travelled merely 60 km only had increased AST levels after lairage. The 60 km road travel period was thus not sufficient to induce changes in the AST concentration immediately after transportation, and the changes only occurred later on during lairage.

The birds that travelled only 60 km to the abattoir had a second blood sample taken from them before transport. This sample had a significantly higher AST concentration ($P = 0.013$) than the first sample (baseline). The reason for this is unknown and could have resulted from the physical stress of handling during weighing and blood sampling at 07:00 earlier that morning (baseline).

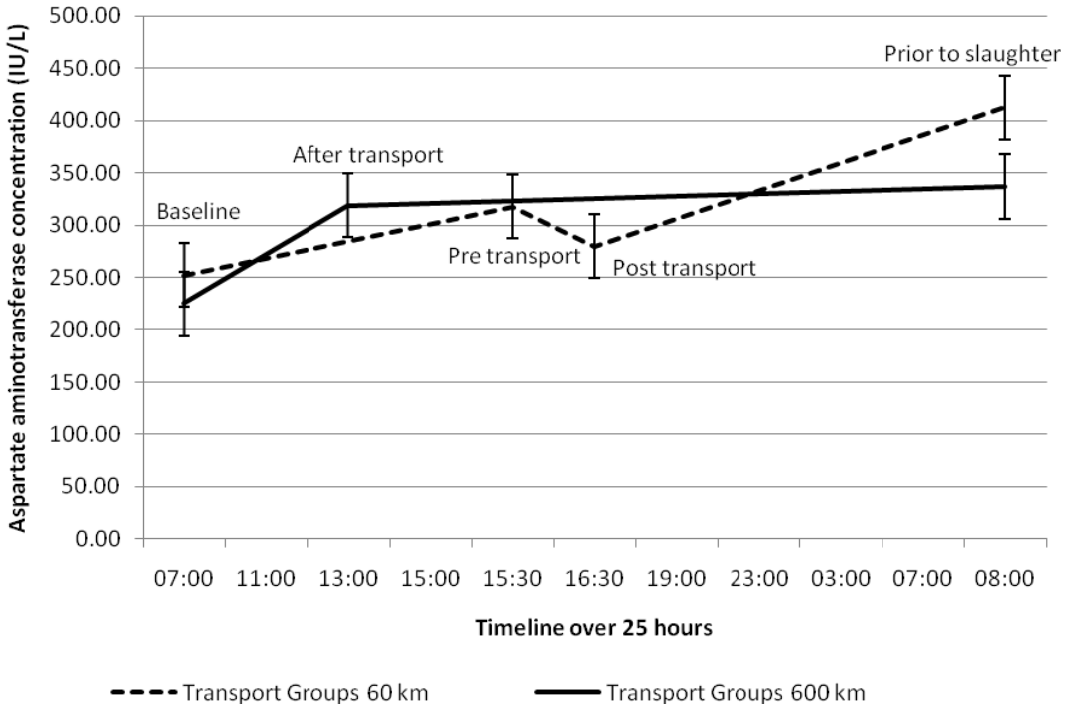

Figure 3.3. Mean aspartate aminotransferase concentration over a period of 25 hours for ostriches that travelled 60 km (n=8) and 600 km (n=8) respectively to the abattoir.

CONCLUSION

The results in this study indicate that no protein catabolism and dehydration took place in the ostriches that were transported and deprived of feed for 24 hours, due to the fact that there was no significant difference between total protein, albumin and globulin plasma concentrations of the two groups of ostriches at any time point during the study. The total protein, albumin and globulin concentrations within each group also remained stable from baseline up to pre-slaughter, indicating that the time effect didn't have a major influence on total protein levels.

In terms of haematological indicators, in the current study, the time effect had the greatest influence on WBC counts. There was a definite increase in the plasma WBC count of both treatments B and C over time from baseline to pre-slaughter levels. The increases in WBC may be an indication that during the *ante-mortem* period, cell damage occurred, that resulted in an inflammation response which in turn led to an increase in the amount of WBCs in the blood of the ostriches that were transported. The H:L ratio is an indication of long term changes in the environment of an animal. In the current study, treatment B was the only group that showed a significant increase in their H:L ratios during transport and it seems as if the longer distance

travelled had this impact on the H:L ratio. However during lairage, the H:L ratio of these birds returned to normal – thus indicating their ability to recover from stress.

During the study the corticosterone levels of the ostriches of treatment B remained stable and no increases were observed over time. However, the major increase in corticosterone concentration seen in treatment C occurred pre-transport, whilst the birds of treatment C were still on the farm. This probably confounded the data and resulted in the corticosterone concentration of group C remaining elevated for the duration of the trial and significantly higher than that of treatment B at all time points except baseline. The fact that the corticosterone increases in treatment C occurred pre-transport probably indicates that these birds suffered from acute stress prior to slaughter rather than stress related to transport. The acute stress response seen prior to slaughter may have been in reaction to an acute stressful episode during this waiting period.

There were significant increases in CK and AST levels in both treatments B and C during the *ante-mortem* period. Both CK and AST levels indicate that all transported birds suffered from physical stress during the *ante-mortem* period and that muscle damage occurred as a result. Further research is required to see whether birds that travelled long distances to abattoirs would be able to restore their plasma CK and AST concentrations to normal levels and in turn lead to the production of better quality meat.

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Chapter 4

EFFECT OF TRANSPORTATION ON OSTRICH (*Struthio camelus*) WEIGHT LOSS AND MEAT QUALITY

ABSTRACT

Ante-mortem stress is known to adversely affect the meat quality of animals. Transportation and lairage of animals, prior to slaughter, also lead to weight loss that affects the economical returns of ostrich producers. There is a need to decrease *ante-mortem* stress of animals during transport and develop management practices to improve transport conditions of ostriches. The purpose of this study was to compare the effect of different transport distances on different meat quality parameters, weight loss, and dressing percentage. Twenty four ostriches were transported for 0, 60 and 600 km respectively before being slaughtered. There was a significant difference in the percentage live weight loss during transport and lairage between the groups that travelled 60 (2.4 ± 2.185 %) and 600 km (8.13 ± 1.156 %) respectively. There was also a significant difference in dressing percentage (farm weight to cold carcass weight) between the two above-mentioned groups. Treatments also had a significant effect on pH_u with the ostriches that didn't travel exhibiting the lowest pH_u (5.77 ± 0.053) and the birds that travelled 600 km having the greatest pH_u (6.11 ± 0.053). No differences were found in shear force and percentage cooking loss between the treatments, although all three treatments differed significantly from each other in drip loss. Significant differences were found for L*, b* and hue angle between the three treatments, whilst there were no differences in a* and chroma values between the treatments. A significant correlation was found between hue angle ($r = 0.528$; $P = 0.008$) and pH_u. Of specific interest to the meat industry, is the fact that at the time of slaughter, meat drip loss and s-CK (chapter 3) was significantly correlated ($r = 0.484$; $P = 0.022$), which indicates that CK levels may be used as a predictor of meat quality prior to slaughter. The results of this study indicate that if ostriches are transported for longer distances to abattoirs, they would lose more live weight and these longer transport distances before slaughter would also have an adverse effect on the meat quality of these ostriches.

INTRODUCTION

Ostrich farming in South Africa was initiated in the 1860s to supply feathers as a fashion item (Smit, 1963). From the 1860s to World War II, feathers were the major source of income for the ostrich industry. However, after World War II, there was an increase in the production of biltong (dried meat similar to jerky) from the meat of ostriches (Smit, 1963). Biltong became the main product generated from ostriches in 1963 when the KKLK (Klein Karoo Landbou Koöperasie) abattoir was established in Oudtshoorn (Smit, 1963; Drenowatz *et al*., 1995). According to Lambrechts and Kruger (2006), ostrich meat has become increasingly popular under consumers as a healthy alternative meat product to red meat. According to Hoffman *et al*. (2005), ostrich meat has a favourable fatty acid profile whilst Girolami *et al*. (2003) reported that ostrich meat is low in cholesterol and fat. Ostrich meat now generates about 60% of the income of ostrich products, whilst leather generates 30% and feathers about 10% (Brand, 2010). Recent figures indicate that South Africa alone produces about 260 000 slaughter birds per annum (Brand, 2010). The primary source of income in the ostrich industry has now shifted to meat (Cloete *et al*., 2002), and more information is consequently needed to improve the quality characteristics associated with ostrich meat (Hoffman *et al*., 2007).

Although ostrich meat is considered as very healthy with a large proportion of polyunsaturated fatty acids, modern consumers are also concerned about the welfare of ostriches and in particular slaughter birds, especially during the *ante-mortem* period (i.e.before the animal is slaughtered) (Lambooij, 1999). The critical factors that could lead to *ante-mortem* stress would be incorrect handling, transport and lairage prior to slaughter. Supermarkets and consumers are also concerned about the quality of the product when purchasing ostrich meat.

Prolonged *ante-mortem* stress can significantly modify the quality of meat (Warriss, 2000). Ostrich meat is perceived as a dark meat with a high ultimate pH (Harris *et al*., 1994). Harris *et al*. (1994) noted that ostrich steaks were significantly drier than beef loin steaks that contained a higher percentage of intramuscular fat. These perceived characteristics of ostrich meat may be attributable to either pre-slaughter stress resulting in dark, firm and dry (DFD) meat (Balog & Almeida Paz, 2007) or to the fact that ostrich meat has a high pigment (myoglobin) content and low levels of intramuscular fat (Harris, 1994).

A*nte-mortem* stress in ostriches can be minimised by reducing stress during capture, transportation and lairage. However, to address the problem of *ante-mortem* stress, the causes of stress during these periods has to be defined and the effects that *ante-mortem* stress has on the product quality and animal welfare quantified. Although relatively little research has been done on the effect of *ante-mortem* stress on meat quality in ostriches, a few authors have investigated the problem (Van Schalkwyk *et al*., 2005; Fasone *et al*., 2005; Hoffman & Lambrechts, 2010). Although there is very little information regarding the transport of ostriches, there are industrial guidelines that deal with transport densities on trucks and lairage design at abattoirs. Many farmers are also of the opinion that their birds loose considerable weight when being transported, particularly when they travel for long distances to abattoirs. There are some farmers in South Africa who transport their birds over a 1 000 km, although it happens only on in exceptional circumstances.

According to Reiner *et al*. (1996), ostriches are prone to stress and are easily startled when they come into contact with humans, leading to an instinctive flight response. Activity, such as handling and transport, is perceived by the ostrich as novelty and causes stress and injury that in turn leads to poor meat quality and economic losses.

This study was conducted to determine the effect of *ante-mortem* stress and different transport distances/periods on weight loss and meat quality in slaughter ostriches. No study to date reported on the effect that different transport distances have on ostrich meat quality. This work will build on some of the earlier work by Van Schalkwyk *et al*. (2005) on lairage times, and the effect it has on meat quality, as well as evaluate the effect of different transport distances on the meat quality.

MATERIALS AND METHODS

Experimental Animals

For this study, 24 South African Black ostriches (*Struthio camelus* var. *domesticus*) were used from the Kromme Rhee experimental farm outside of Stellenbosch. The ostriches used in the trial were divided into three groups of eight birds each, and each group consisted of four male and four female ostriches. The experimental birds were approximately 10 months old, and were raised in the feedlot on the Kromme Rhee farm. The trial was conducted from 4 to 6 May 2009. All the birds received the same finisher diet until slaughter

Experimental design

The experimental design of this trial is a completely randomised design with three treatments (transport distance) and eight birds per treatment. Ethical approval for the study was obtained from the Stellenbosch University Sub-committee B Animal Research Ethics committee (reference number, 2009B03003).

Transport and Slaughter

Treatments A, B and C were the three groups of ostriches that travelled 0, 600 and 60 km respectively to the abattoir before slaughter. The ostriches used in the trial were familiar with humans and handling by humans due to the fact that they were raised in a feedlot and were in contact with people on a daily basis.

Each ostrich of treatment B and C was weighed, before the birds of treatment B were loaded onto a transport trailer from 07h00 onwards. The birds of treatment B were transported from 08h00 to 13h00 on a 600 km circular route during which the birds were visually monitored. The birds of treatment B were deliberately transported to the abattoir via a longer route for the purpose of the study. On their arrival at Swartland abattoir at 13h00, (treatment B) each ostrich was individually offloaded and weighed before being placed into one lairage pen, to overnight, with access only to water. During lairage, the ostriches were monitored by a member of the research team, to determine their behaviour. The same trailer that transported the birds of treatment B then travelled back to Kromme Rhee to fetch the birds of treatment C, which had remained in their paddocks until 15h00, with access only to water, while the birds of treatment B (600 km) were being transported. From 15h00, the birds of treatment C were individually weighed before being loaded onto the truck at Kromme Rhee farm. The ostriches of treatment C were transported from 15h30 for 60 km, directly to the Swartland abattoir where they arrived at 16h30. The two groups of ostriches were transported according to the guidelines of the LWCC (Livestock Welfare Coordinating Committee) with a minimum floor space of 0.5 m^2 per bird. At their arrival at Swartland abattoir, each bird in treatment C was individually offloaded and weighed and all the birds of treatment C placed in a lairage pen adjacent to the birds of treatment B with access only to water. Both the treatment B and C groups remained in lairage over night as is normal practice at abattoirs and were monitored until 22h00 that evening by a member of the research team.

The birds that were transported (B and C) travelled in a specially designed trailor for the transportation of ostriches. The trailer consisted out of two compartments whilst there were two female and two male birds in every compartment with an average floor space of 0.5 m^2 per bird. The trailor was pulled by a 4×4 vehicle whilst the trailor's floor had steel grids. During transport of the birds in treatment B the driver, who was

experienced in transporting ostriches, was only allowed to stop twice to refuel the vehicle. The driver was not allowed to stop when he transported the birds in treatment C. At all times the driver had to keep a maximum speed of 100 km/h.

At the abattoir birds were handled by experience handlers and placed into lairage pens adjacent to the abattoir. Birds had access to water whilst in lairage whilst the floor of the lairage pen was concrete. Both these groups were slaughtered at Swartland commercial abattoir in Malmesbury, the day after they were transported. The birds were slaughtered according to normal ostrich abattoir practises. The trachea, stomachs and intestines of each bird were identified and placed together in a bucket to record their weights their weights the same afternoon.

The following morning from 08h00, the ostriches (treatment B and C) were again individually weighed before slaughter. The live weight losses and the percentage weight loss were then calculated during each phase. The ostriches of treatment B were slaughtered first, followed by the birds of treatment C. The ostriches were slaughtered according to normal ostrich abattoir practice. During slaughter, all the organs of the viscera were removed, as well as the tracheas, skins, feathers, heads, feet, excess fat, and wing points. The weights of the warm and cold carcasses were recorded before the bruised meat on each carcass was removed from the carcasses. The stomachs and intestines of each bird were removed, marked and placed in separate buckets during slaughter to record the respective digestive system weights of each ostrich at a later stage. The full and empty weights of each of the sections of the digestive system were recorded and the percentage contents in each of the sections was then determined by taking the difference in weights of the full and empty digestive tract section as the amount of digesta present in the particular section at slaughter.

All carcasses were marked with a plastic tag with the bird ID displayed on the tag to distinguish each individual ostrich carcass in the abattoir. Whilst still on the slaughter line, pH and temperature readings (pH_1 and $temp_1$) were taken 1 hour *post-mortem* from each ostrich's right big drum (*M. gastrocnemius*) muscle in the B and C treatments, where after the carcasses were placed in a chiller. Prior to measuring, the Testo 205 pH meter (Testo AG, Germany) was calibrated.

The control group of ostriches (Group A; n = 8) was maintained for 6 days at the Mariendahl Experimental Farm outside Stellenbosch, and was slaughtered on the farm without being transported prior to slaughter. The birds were moved from the Kromme Rhee farm 6 days before they were slaughtered. Slaughter birds from the Group A were of the same age and raised in the same manner, on Kromme Rhee experimental farm, as the other two treatment groups (Group B and Group C). The control group of birds was slaughtered on the farm by a research team of the University of Stellenbosch and workers of the Kromme Rhee experimental farm - the slaughter plant was adjacent to their feedlot paddock. The birds of the control treatments' feed was only removed prior to slaughter. The birds were fed the same finisher diet up to slaughter than the birds in treatments B and C.

Each bird in treatment A was individually weighed prior to slaughter, and then electrically stunned to render them unconscious. Their throats were slit using a sharp knife. Once the bird had ceased kicking (and was therefore considered dead), they were hung onto hooks to drain the blood from the carcass. When the bird was fully exsanguinated, it was plucked, skinned and and incision made to remove the abdominal cavity contents. All visceral organs, with exception of the lungs, were removed. The trachea, stomachs and intestines of each bird were identified and placed together in a bucket to record their weights that same afternoon. The skin, head and feet were then removed from each ostrich and their weights recorded. Cuts were made through the Achilles tendon of each carcass from which it was then hung from a hook on a wooden crossbar to determine the weight of the carcass.

pH measurements

After each bird was slaughtered, pH and temperature (pH_1 and $temp_1$) was recorded in the right big drum muscle (*M.gastrocnemius*), 1 hour *post-mortem*. The carcasses were then placed in a cooler (4^{o}C) and transferred to the research meat laboratory where the carcasses were placed in a chiller until further processing.

Ultimate pH (pH_u) and temperature measurements were again taken 24 hours *post-mortem* in the chiller. Muscle samples were subsequently removed from the right *M. gastrocnemius* and placed in plastic bags, numbered with the corresponding ostrich's ID, and taken back to the University of Stellenbosch's meat laboratory where further meat analyses were performed on the meat.

Physical analysis

One large meat sample was taken from the right *M. gastrocnemius* of each carcass and used to determine drip loss, cooking loss, Warner-Bratzler shear force values and colour measurements on the meat of the 24 birds in the three treatments. These samples were analysed according to the method described by Honikel (1998).

The percentage drip loss was determined by hanging individually-weighed samples (sample weight between 50g and 100g) in inflated polythene bags for 24 hours at ± 4^{o}C in a chiller room. Care was taken that the samples did not touch the sides of the inflated bags. After 24 hours, the samples were removed and weighed, and the percentage drip loss calculated as the amount of weight lost from the sample during the 24 hours the sample spent in the polythene bag. The percentage cooking loss was calculated by weighing the meat samples (50-100 g) before they were cooked. The samples were then placed in polythene bags in a water bath at ± 80^{o}C for 50 minutes. The samples were then removed from the water bath, the water drained from the bags and the samples (still in the bags) cooled under running water to ± 20^{o}C. After cooling, the samples were removed from the bags and patted dry with tissue paper and subsequently weighed. The percentage cooking loss was calculated as the amount of weight lost by each sample during the cooking period.

The cooled meat samples used in the above-mentioned cooking-loss procedure was then used to determine tenderness using a Warner-Bratzler device, with a load of 2.000 kN, attached to a Instron (Model 4444) Testing Instrument. Five cylindrical core samples of 1.27cm diameter each were cut from each cooked piece of muscle (5 pieces from each bird) at random locations on the cooked piece. Maximum Warner-Bratzler shear force values were recorded by cutting the cylindrical core of cooked muscle perpendicular to the longitudinal orientation of the muscle fibres at a crosshead speed of 200 mm/min. An average shear force value (N) was then calculated for each bird. Care was taken to avoid cylindrical core samples that contained visible connective tissue that could maybe influence shear force results.

Statistical analysis

Data was analysed using Statistica v.8. Analyses included ANOVA with the Fisher LSD (least square difference) test as the post hoc test. The ages of the birds were not known, and the gender of the birds was neglected which meant that the only main effect considered, when analysing the data, was the treatment effect. The differences between the groups of birds (i.e. treatments) were, where appropriate, tested by means of the null hypothesis (H_o), with H_o: $\mu_1 = \mu_2 = \mu_3$ and the alternate hypothesis (H_a) is that H_a: $\mu_1 \neq \mu_2 \neq \mu_3$. Data are presented as means and standard errors. A p-value of $P < 0.05$ was accepted as statistically significant**.** A Pearson correlation coefficient was also calculated for the pH_u vs. the shear force, drip loss and cooking loss percentages. A Pearson correlation coefficient was also calculated for the percentage drip loss *vs.* the serum concentration of post-slaughter creatine kinase (see chapter 3). Proc GLM was also performed on the pH data. In the case of the pH values, all the pH values of all the birds were also divided into categorical data according to the pH value of the birds. The pH categories were pH > 6.2, 6 - 6.2, 5.8 - 5.99 and < 5.8. The percentage of birds that fell into each category was noted. This was done because individual birds differ in their response to stress and for abattoirs to determine the proportion of birds that has high (extreme) pHes that could result in DFD meat.

RESULTS AND DISCUSSION

Live weight loss

Live weight loss during transportation and lairage is a big concern to producers due to the high proportion of live weight the digestive tract contributes (Romans *et al*., 1994). Ostrich farmers in South Africa are aware of the economic importance of live weight loss and are of the opinion that birds lose considerable weight when being transported, particularly over long distances. Farmers in South Africa are of the opinion that that birds that travel for longer distances lose more weight. They are also of the opinion that birds do not defecate during travel. According to Skadhauge *et al*. (1984) the relative length of the digestive system of an adult ostrich is approximately 23.95m, with the feed retention time about 40 hours (Swart, 1988). The amount of ingesta present in the digestive tract is dependent upon the amount of time birds are kept off feed and water (Morris *et al*., 1995a). In this investigation, the ostriches that were not transported (control group) had an average digestive tract weight of 11.55 ± 0.606 kg and an average live weight of 88.75 ± 2.624 kg prior to slaughter. Thus, the average digestive tract weight accounts for 13.01% of the total weight of the ostrich prior to slaughter without being transported.

The live weights of treatments B and C are presented in Table 4.1 at three different intervals during the trial. Statistically there were no differences between the live weights of the ostriches in treatment B and C at each of the intervals. The general trend was for the live weight of each group to decrease over time from pre-transport to just prior to slaughter. This was expected due to the fact that the birds in both groups were deprived of feed for approximately 24 hours.

Table 4.1 also shows the mean weight loss and percentage weight loss of treatments B and C after transport, as well as the mean weight loss of each group after transport and lairage. There was a difference ($P = 0.001$) in the mean weight loss and percentage weight loss ($P = 0.001$) between treatments B and C after transport – in fact the live weight of ostriches that travelled the shorter distance (treatment C) remained the same. After lairage, the total weight loss as well as the percentage weight loss between treatments B and C, were still significantly different.

Van Schalkwyk *et al*. (2005) reported a weight loss of 1.04 ± 0.51 and 3.23 ± 0.56 kg in birds that were kept off feed for 24 and 56 hours respectively whilst the ostriches used in the trial by Van Schalkwyk *et al*. (2005) were of a similar weight than the birds used in the current study. The weight loss reported by Van Schalkwyk *et al*. (2005) is similar to the weight loss seen in the ostriches of treatment C in the current trial. However, the birds in the study done by Van Schalkwyk *et al*. (2005) were transported only 10 km to the abattoir. It must be kept in mind that both groups in the current trial, were kept off feed for the same period prior to slaughter with only the distances they travelled to the abattoir varying. This finding supports the opinions of ostrich farmers that birds that travel further have greater live weight losses than birds that travel for shorter distances, regardless of the amount of time they are kept off feed. This observation is even more compelling given the fact that during lairage, there were no differences between treatments B and C, based on mean

weight loss and percentage weight loss. The act of transportation thus has the largest effect on weight loss in ostriches in the current trial.

Table 4.1
Mean live weights (kg) and live weight losses (kg and %) of the ostriches in treatments B and C at different intervals during the trial.

Live weight and Weight lost	Weights (Mean ± s.e.)		P < |t|
	Transport Groups		
	Treatment B (600 km)	Treatment C (60 km)	
Weight before transport (kg)	93.88 ± 4.038	92.75 ± 4.038	0.847
Weight after transport (kg)	89.19 ± 4.173	93.16 ± 4.173	0.512
Weight loss during transport (kg)	4.69 ± 0.896	- 0.41 ± 0.896	0.001
Weight loss during transport (%)	5.11 ± 1.26	-0.47 ± 0.636	0.001
Weight after lairage (kg)	86.33 ± 3.405	90.01 ± 3.405	0.457
Weight loss during lairage (kg)	2.86 ± 0.889	3.15 ± 1.902	0.893
Weight loss during lairage (%)	3.14 ± 0.937	2.83 ± 2.267	0.9
Total weight loss after transport and lairage (kg)	7.55 ± 1.528	2.74 ± 1.528	0.043
Total weight loss after transport and lairage (%)	8.13 ± 1.156	2.4 ± 2.185	0.036

The mean weights (and the percentage contents) of each of the different sections of the digestive tract of the three groups of ostriches in the current trial are presented in Table 4.2. These values were determined to see where the loss in live weight occurred within the digestive system. There were no significant differences between the mean total weights of the digestive tracts and each section of the digestive tracts between the three groups of ostriches. However, the control group had a significantly greater amount of ingesta in the digestive tract compared to treatment C (P = 0.003) and treatment B (P = 0.015), whilst treatment B and C didn't differ (P = 0.452) in their percentage digestive tract contents. This is expected because of high levels of faecal output during transportation and because the two groups that travelled had a longer period of feed deprivation before they were slaughtered.

There are few studies in literature that report on the weights of the digestive system of the ostrich. The mean total digestive tract weight (10.88 ± 0.3 kg) in the current study was much greater than the alimentary tract weights reported by Van Schalkwyk *et al*. (2005), who reported values of 0.653 ± 0.041 and 0.663 ± 0.036 kg respectively for ostriches that were deprived of feed for 24 and 56 hours. Morris *et al*. (1995a) reported

values of 8.29 kg ± 4.11 kg for the full viscera and 2.15 ± 0.09 kg for the empty gizzard and crop of ostriches slaughtered between 10 and 14 months of age. The values of Morris *et al*. (1995a) are similar to the range of values reported in the current study for digestive tract weights. There were no differences in the percentage contents of the small intestines between the three groups of ostriches. The only significant difference (P = 0.007) in large intestine contents was between the control treatment (53.45 ± 3.187 %) and treatment C (39.88 ± 3.187 %). There were also differences between the percentage contents of the stomach between the control group and the two groups that were transported prior to slaughter, with the control group having greater amounts of contents in the stomach than treatments B and C. Van Schalkwyk *et al*. (2005) reported lower full stomach weights than in the current study. They found that the full stomach weights of birds deprived of feed for 24 and 56 hours were 0.51 ± 0.023 and 0.49 ± 0.023 kg respectively, whilst the current study reported a mean full stomach weight of 1.36 ± 0.076 kg for the birds in treatment B and C. When the weight of the digestive tract was calculated as a percentage of the live weight just before slaughter, there were no differences between the three groups. However, the birds of treatment A had the highest percentage contents in their (see Table 4.2) digestive tract when compared to treatment B and C.

In the current trial, the ostriches of treatment B clearly lost the most weight during transport and lairage. However, these losses are not reflected in the relative weights and percentage contents of each of the three groups' individual digestive tract sections. However, the control group had the heaviest digestive tract weights along with the heaviest relative digestive tract sections in all the cases, mainly due to the fact that these birds weren't deprived of feed for as long as the other two groups. However, although the birds of treatment B had a significantly greater live weight loss than the birds of treatment C, there were no differences in the relative digestive tract weights and percentage contents between these two groups (Table 4.2). This is an indication that a proportion of the live weight loss in the birds of treatment B, resulted from weight loss in the muscles of these birds. Observations made during transportation also bore evidence that birds urinated and defecated during transport.

Table 4.2

Mean weights (kg) and percentage contents of the digestive systems of the control and treatment B and C ostriches, as well as the mean weights (kg) and percentage contents of each section in the digestive system of the three treatment groups.

Digestive system weights and % weights	Transport Groups (Mean weights ± s.e.)			Comparison of Transport Groups P < |t|		
	Control Group (0 km)	Treatment B (600 km)	Treatment C (60 km)	Control vs Treatment B	Control vs Treatment C	Treatment C vs Treatment B
Total weight of digestive tract	11.55 ± 0.509	10.7 ± 0.509	10.41 ± 0.509	0.250	0.128	0.694
% Contents in digestive tract	44.22 ± 1.832	37.36 ± 1.832	35.38 ± 1.832	0.015	0.003	0.452
Weight of stomach	1.47 ± 0.076	1.36 ± 0.076	1.36 ± 0.076	0.293	0.298	0.991
% Contents of stomach	49.75 ± 3.328	14.92 ± 3.328	20.91 ± 3.328	0.000	0.000	0.217
Total weight of gizzard	3.12 ± 0.18	2.79 ± 0.18	2.94 ± 0.18	0.197	0.472	0.555
% Contents of gizzard	40.98 ± 2.88	46.61 ± 2.88	51.32 ± 2.88	0.18	0.02	0.26
Weight of small intestine	1.69 ± 0.123	1.52 ± 0.123	1.44 ± 0.123	0.365	0.178	0.646
% Contents in small intestine	15.22 ± 4.869	6.73 ± 4.869	-2.69 ± 4.869	0.231	0.017	0.186
Weight of large intestine	5.27 ± 0.285	5.03 ± 0.285	4.67 ± 0.285	0.563	0.156	0.387
% Contents of large intestine	53.45 ± 3.187	45.9 ± 3.187	39.88 ± 3.187	0.109	0.007	0.196

Dressing percentage and Carcass weight

The mean dressing percentage and carcass weight of the two respective groups of ostriches that were transported are depicted in Table 4.3. In this case only the 600 km and 60 km groups' dressing percentages and carcass weights were compared because of the difference in slaughtering practices between the Swartland abattoir and the research staff of the University of Stellenbosch who slaughtered the control group of birds. It was impossible to obtain the services of the Swartland abattoir's personal for the slaughtering of the ostriches on the farm in Stellenbosch due to their work commitments at the abattoir. The only significant differences in dressing percentages between the 2 groups of ostriches, were between the farm weight to warm carcass weight (P = 0.004), as well as the farm weight to cold carcass weight (P = 0.003). In both the above-mentioned cases, treatment C exhibited a significantly greater dressing percentage than the birds of treatment B. This can possible be ascribed to the greater live weight loss that occurred in the ostriches of treatment B (Table 4.2).

There were no significant differences in abattoir to warm carcass and abattoir to cold carcass yield percentages between the two groups. The cold carcass dressing percentages in the current study agree with Balog and Almeida Paz (2007) who reported a cold carcass yield of 51 % for birds slaughtered at an age of 12 to 14 months. Morris *et al*. (1995a) however, reported a greater percentage carcass weight (58.59 %) for ostriches slaughtered between 10 and 14 months. There were no differences between the warm and cold carcass weights of the two groups that were transported although in both cases treatment C had about a 2 kg greater carcass weight than treatment B.

Table 4.3

Mean dressing percentages and carcass weights of treatments B and C.

Dressing Percentage and Carcass weight	Transport Groups (Mean % ± s.e.)		Comparison of Transport Groups $P < \|t\|$
	Treatment B (600 km)	Treatment C (60 km)	
Warm carcass (kg)	44.99 ± 1.920	47.24 ± 1.920	0.421
Cold Carcass weight (kg)	44 ± 1.878	46.06 ± 1.878	0.450
Farm weight to warm carcass (%)	47.92 ± 0.653	51.05 ± 0.653	0.004
Abattoir weight to warm carcass (%)	52.17 ± 0.766	52.43 ± 0.766	0.812
Farm weight to cold carcass (%)	46.86 ± 0.575	49.77 ± 0.575	0.003
Abattoir weight to cold carcass (%)	51.03 ± 0.782	51.12 ± 0.782	0.934

pH

Due to the fact that ostriches are very susceptible to stress and have unique meat characteristics such as high myoglobin content, low intramuscular fat content and a relatively high ultimate pH, the meat of ostriches can easily be perceived as DFD (Harris *et al*., 1994; Reiner *et al*., 1996). The higher ultimate pH in ostrich meat also leads to a reduction in the shelf life of ostrich meat products (Lawrie, 1998).

The extent of pH decline in meat *post-mortem* is generally accepted as a measure of *ante-mortem* stress in animals (Mounier *et al*., 2006). *Ante-mortem* stress leads to glycogen depletion prior to slaughter, resulting in minimum lactic acid production during the *post-mortem* period. When insufficient lactic acid is produced in the muscles *post-mortem*, this usually leads to the production of DFD meat with a high ultimate pH (pH_u) (Sales & Mellet, 1996). Newton and Gill (1981) defined DFD meat as meat with an ultimate pH above 6 ($pH_u > 6$). DFD meat has the potential to spoil more rapidly than meat with lower pH_u (Newton & Gill, 1981). A high pH_u is a common phenomenon in ostrich muscles regardless of pre-slaughter handling and stunning (Sales & Mellet, 1996). In the current study, pH_u was measured at 24 hours *post-mortem* (Table 4.4). There was a difference ($P = 0.028$) in pH_u between treatment B and C, as well as the control and treatment B ($P = 0.000$). The control and treatment C groups also had a different pH_u when compared to each other ($P = 0.050$). In groups B and C there was a tendency for the pH values to decrease over 24 hours (Figure 4.1), whilst the control group had similar pH values at 1 and 24 hours *post-mortem*. The pH_u values of the ostriches in this study correspond to the values of Sales and Mellet (1996) (who reported pH_u values between 5.8 and 6.2) and the control group of Fasone *et al*. (2005) ($pH_u = 5.94 \pm 0.08$). The other group of ostriches in the study by Fasone *et al*. (2005) had a significantly higher pH_u ($pH = 6.96 \pm 0.17$) than the pH_u in the current study. This is probably due to the fact that this group of ostriches suffered much greater stress prior to slaughter than any of the other studies mentioned. These birds (the birds with an pH_u of 6.96 in the trial of Fasone *et al*., 2005) were classified as being stressed prior to slaughter because they suffered from broken legs during transport or were insufficiently stunned prior to slaughter. The stress endured by these birds ((stressed group in the study by Fasone *et al*. (2005)) is probably much greater and painful than the stress caused by transportation in this investigation.

Table 4.4

Mean pH_1 and pH_u values measured at 1 and 24 hours *post-mortem* for the groups of ostriches that travelled different distances prior to slaughter.

Transport Groups	Mean pH (± s.e.)		Comparison of Transport Groups	P < |t|	
	pH_1	pH_u		pH_1	pH_u
Control Group (0 km)	5.80 ± 0.053	5.77 ± 0.053	Control Group vs Treatment C	0.000	0.05
Treatment C (60 km)	6.30 ± 0.053	5.93 ± 0.053	Control Group vs Treatment B	0.000	0.000
Treatment B (600 km)	6.37 ± 0.053	6.11 ± 0.053	Treatment C vs Treatment B	0.338	0.028

The birds of treatment C had the biggest decrease in pH, from 6.3 directly after slaughter (pH_1) to 5.93, 24 hours later (pH_u) (Figure 4.1), indicating that the birds of treatment C had a better chance to restore their muscle glycogen levels during lairage or that these birds did not use their glycogen stores during transport. This could well be an indication that these birds did not suffer as much physical stress as their counterparts that travelled 600 km.

The birds in treatment B's glycogen reserves were more depleted during their longer transit period and these birds struggled to restore glycogen reserves during lairage, leading to moderate DFD meat, when the pH's after 1 and 24 hors were of treatment B and C were compared to each other. The act of balancing and keeping upright during transport is probably the major contributing factor to the energy and glycogen reserves being depleted during transport and even more so if the animals travel greater distances. During the regular visual monitoring of the ostriches in transit, it became clear that during travel all the birds remained standing, with some of the birds immediately sitting down when the vehicle stopped (for example during refuelling of the vehicle). Another factor that could also play a role in the greater decline of pH, over 24 hours *post-mortem* of the ostriches in treatment C, is the fact that these ostriches had a significantly higher plasma corticosterone concentration, after slaughter, than the ostriches of treatment B (see Chapter 3). Plasma corticosterone concentration is an indication of short term changes in the environment of an animal (Gross & Siegel, 1983). This may indicate that the ostriches from treatment B may have acclimatised to the stress of transport although they didn't have time to replenish their glycogen reserves. The higher corticosterone levels of the birds in treatment C could have elevated the blood glucose levels prior to slaughter which in turn resulted in greater lactic acid production *post-mortem* of the birds in this treatment.

Corticosterone exerts a hyperglycaemic effect in ostriches during periods of stress, which results in the release of glucose to aid the animal when it will require sufficient energy for a fight or flight type response (Leche *et al.,* 2009). The greater plasma corticosterone concentration observed in the ostriches of treatment C could well have been due to psychological stress rather than physical stress.

The decline of pH in muscles is dependent upon the available glycogen remaining in the muscles *post-mortem*. The breakdown of glycogen will cause lactic acid build-up in the muscles and acidify the meat as previously discussed. The relationship between elevated pH_u and physical stress due to transport was also observed in emus by Berge *et al*. (1997). It is also known that birds respond differently to stress. The pH_u values of all 24 ostriches were not normally distributed but when both non-parametric and parametric tests were performed on the data, it gave the same results i.e. indicating that the pH_u of the three treatments in the current study did differ. A Proc GLM was therefore performed on the data. Table 4.5 depicts the pH categories and the percentage of pH values that fall into each of the three groups of ostriches at each of the pH time points.

It is evident that the data of the pH_us of all 24 ostriches are not evenly distributed. In the current study, 37.5 % of the ostriches in treatment B (3 of the 8 ostriches) exhibited pH_u values within the range considered to produce extreme DFD meat, with pH_u values higher than 6.2. The other 5 ostriches in treatment B all exhibited pH_u values associated with intermediate DFD meat, with their pH_u values ranging from 5.8 to 6.2. These pH_u values of the birds in treatment B are in stark contrast to the pH_u values of the control group and treatment C. The control group and treatment C had 0 % and 12.5 % of animals in their groups, respectively that had pH_u values higher than 6.2; also classified as extreme DFD meat (Table 4.5). The control group (87.5%) and treatment C (37.5%) contained the most birds exhibiting normal pH_u values of below 5.8.

Table 4.5

Summary of the pH categories of the $pH_{(1\ hour)}$ and pH_u of each of the three groups of ostriches that travelled different distances prior to slaughter.

Time point	pH category	Treatment group			Grand Total
		Control Group	Treatment C (60 km)	Treatment B (600 km)	
1 Hour *post-mortem* ($pH_{(1\ hour)}$)	pH >6.2	0.00%	75.00%	87.50%	54.17%
	pH 6 - 6.2	0.00%	25.00%	12.50%	12.50%
	pH 5.8 - 5.99	37.50%	0.00%	0.00%	12.50%
	pH <5.8	62.50%	0.00%	0.00%	20.83%
Chiller (24h) (pH_u)	pH >6.2	0.00%	12.50%	37.50%	16.67%
	pH 6 - 6.2	0.00%	25.00%	37.50%	20.83%
	pH 5.8 - 5.99	12.50%	25.00%	25.00%	20.83%
	pH <5.8	87.50%	37.50%	0.00%	41.66%

Water-holding capacity and tenderness

The term 'water-holding capacity' (WHC) is described as 'the ability of meat to retain water during the application of external forces' such as cutting and heating (Swatland, 1994). The water-holding capacity of meat can also influence other characteristics of meat such as juiciness, colour and tenderness (Sales & Horbanczuk, 1998).

There were significant differences in drip loss between the three groups of ostriches in the current trial, although there were no differences in cooking loss between the three groups (Table 4.6). Van Schalkwyk *et al*. (2005) found no differences in the cooking loss and drip loss values of the control and stressed groups of ostriches. The mean drip loss (0.91 ± 0.48 %) in the current trial was lower than the drip loss values observed by Van Schalkwyk *et al*. (2005) who found that the drip loss in the control and stressed groups of ostriches were 2.18 ± 0.24 % and 1.88 ± 0.26 % respectively. Hoffman *et al*. (2007) also found a higher percentage drip loss (2.1 ± 0.79) for South African Black ostriches in the *M. gastrocnemius.* Unlike the current trial, Fasone *et al*. (2005) did find a significant difference in cooking loss between a control group (not stressed) and a stressed group of ostriches.

Fasone *et al*. (2005) reported cooking loss values of 23.46 ± 3.86 % and 19.46 ± 4.97 % respectively for the control and stressed groups of ostriches, which is lower than the mean cooking loss values (35.826 ± 5.976

%) in the current study. However, Hoffman *et al*. (2007) reported similar cooking loss values (38.0 ± 1.29) to the current study, whilst Van Schalkwyk *et al*. (2005) reported results of 30.8 ± 1.2 % and 29.1 ± 1.3 % respectively for a control and stressed groups of ostriches.

A difference (P = 0.000) was found in drip loss between the two groups that were transported, with the birds of treatment C having a higher mean drip loss (1.36 ± 0.068 %) than the birds of treatment B (0.97 ± 0.068 %). This finding is consistent with the fact that the birds of treatment B had a higher mean pH_u than the birds of treatment C, since an increase in pH_u is known to cause an increase in the WHC of the meat, which would thus lead to a decrease in the amount of drip loss. The control group that was not transported, and supposedly had the least amount of physical and mental stress, had the least amount of drip loss.

Because transport is a physically demanding factor for animals, it can lead to muscle damage. This muscle damage is commonly observed in the blood of animals with the elevation of plasma enzymes such as creatine kinase and aspartate aminotransferase (see Chapter 3), due to increased permeability of the muscle membranes during periods of physical stress such as transport (Warriss *et al*., 1995). In the current study, a positive correlation between the plasma CK (after stunning and exsanguination) and the percentage drip loss (P = 0.022; r = 0.484) in the meat was noted (Figure 4.1). To our knowledge, this is the first study to correlate this enzymatic evidence of cell membrane damage to changes in meat quality of ostriches.

Warriss *et al*. (1998) observed that pigs with the highest levels of CK after stunning and transportation also had the highest percentage of drip loss and the lowest water-holding capacity. The observation by Warriss *et al*. (1998) is in accordance with the results found in the current study. Although there were no statistical difference in the concentration of plasma CK before slaughter in each of the two groups of ostriches that were transported 60 and 600 km respectively, there were significant differences (P = 0.0002) in the amount of drip loss between the two treatments. Treatment C had the greatest amount of drip loss (1.36 ± 0.68 %) whilst treatment B had significantly less (0.97 ± 0.68 %) drip loss in the *M. gastrocnemius.* The expectation would be the opposite; that the birds that travelled 600 km would exhibit the greatest amount of drip loss, due to the fact that the birds of treatment B travelled much further and encountered more physical strain on their muscles. However, in the current study, group B had a longer recovery time than group C, possibly allowing cells with minor damage to have been repaired by the time of slaughter. This would explain the difference in drip loss, which is also further elucidated at the hand of the mean ultimate pH (pH_u) of each of the two groups.

A high ultimate pH leads to meat having a greater water-holding capacity (Offer & Trinick, 1983). The birds of treatment B had a significantly greater ultimate pH than the birds of treatment C and this may have accounted for the decreased amount of drip loss seen in the meat of the ostriches that travelled 600 km. Thus it seems that the amount of physical stress may have led to glycogen depletion, resulting in substances (such as CK) leaking from cell membranes and ultimately influencing the pH of meat – the major determining

factor in the amount of drip loss. Since CK levels correlated with magnitude of drip loss, this suggests that CK may be used as a pre-slaughter predictor of meat quality.

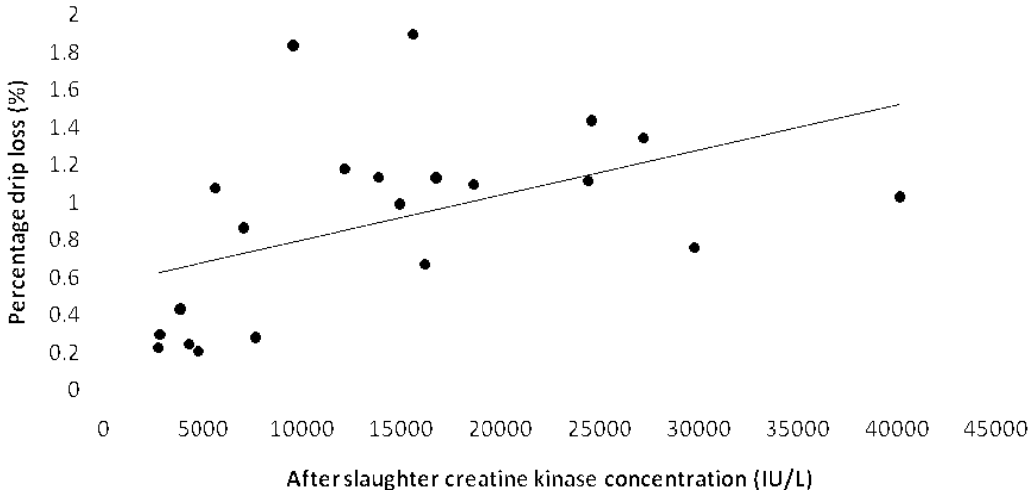

Figure 4.1 Linear correlation (r = 0.484, P < 0.05) between the percentage drip loss and the creatine kinase concentration after slaughter in all the ostriches that travelled to the abattoir (independent of travel distance).

There were no significant correlations between pH_u and the two water-holding capacity characteristics, cooking and drip loss (Table 4.6). The majority of water present in meat is found between the spaces of the thick and thin filaments, whilst the interfilament spacing of the meat varies and is dependant on the pH of the meat (Offer & Trinick, 1983). Thus, if changes in parameters such as pH would occur, it would affect the interfilament spacing and ultimately have an effect on the degree of water-holding capacity and exudate formation. According to Hoffman *et al*. (2005) the WHC of meat tends to be stronger if the pH_u is higher because the filament fibres will be tightly packed together creating a barrier for the diffusion of water. Although there was no correlation between cooking loss and pH_u in the current study, the results reported by Fasone *et al*. (2005) indicate that there is a relationship between the amount of cooking loss and the pH_u. They found that ostrich meat with a pH_u of 6.95 ± 0.17 had a significantly lower cooking loss (19.46 ± 4.97 %) than meat with lower pH_u (5.94 ± 0.08) which had a cooking loss of 23.46 ± 3.86 %.

Tenderness is one of the most critical factors relating to meat quality. There were no differences in shear force values between the three groups of ostriches in the current study (Table 4.6). Fasone *et al*. (2005) found that stressed ostriches had significantly higher shear force values than ostriches that were not stressed, whilst Van Schalkwyk *et al*. (2005) found no differences in Warner-Bratzler shear force values between a stressed and a control group of ostriches. There does not seem to be consensus on the effect pH_u has on the tenderness of meat. Some authors report meat with a high pH_u to be more tender (Silva *et al*., 1999) whilst others report meat with a high pH_u to be less tender (Wulf *et al*., 2002). According to Purchas (1990), there seems to be a curvilinear relationship between the ultimate pH and the tenderness of

cooked meat. He observed that the tenderness decreased as the pH_u increased from 5.5 to 6.0 and that the tenderness increased again when there was an increase in pH_u above 6.0 in the meat. Yu and Lee (1986) observed a similar curvilinear relationship between pH_u and tenderness, with meat having an intermediate pH_u (5.8-6.3) being less tender than meat with a pH_u higher than 6.3 or lower than 5.8. They attributed the increased tenderness of the meat, as the pH_u increased from 6 to 7, to the greater calpain activity which is at a maximum at a neutral pH. The increased tenderness of the meat as the pH_u decreased from 6 to 5 was attributed to the enhanced protease activity at an acidic pH_u.

Table 4.6

Mean drip loss, cooking loss and Warner-Bratzler shear force values as measured in the *M. gastrocnemius* of the three groups of ostriches that travelled different distances.

Meat Quality Parameters	Transport Groups (Mean ± s.e.)			Comparison of Transport Groups P < |t|		
	Control Group (0 km)	Treatment B (600 km)	Treatment C (60 km)	0 vs 60 km	0 vs 600 km	60 km vs 600 km
Drip loss (%)	0.40 ± 0.068	0.97 ± 0.068	1.36 ± 0.068	0.000	0.000	0.050
Cookingloss (%)	32.50 ± 2.017	36.91 ± 2.017	38.06 ± 2.017	0.064	0.137	0.692
Shear force (N)	44.51 ± 3.597	42.74 ± 3.597	38.41 ± 3.597	0.244	0.732	0.403

The curvilinear relationship of pH_u and tenderness, described by Purchas (1990), may be the reason why there was no linear correlation between the pH_u and tenderness in the current study (Table 4.8). Fasone *et al*. (2005) found a relationship between the ultimate pH and tenderness when they reported that ostriches with a significantly higher pH_u ($P < 0.001$) produced meat with considerably lower shear force values ($P < 0.01$). Although Van Schalkwyk *et al*. (2005) found significant differences in pH_u between a control group of ostriches (not stressed) and a stressed group of ostriches ($P = 0.016$) they could not find differences in shear force values of the two groups, although there was a tendency for the shear force values to decrease as the pH_u increased.

When the 24 ostriches in the current trial were divided into two groups, namely those with pH_u values below 6 (normal pH) and those with pH_u values greater than 6.0 (DFD pH) the ANOVA revealed that there were differences ($P = 0.009$) in shear force values between the two groups. The group of ostriches within the normal pH range ($pH < 6$) had a mean Warner-Bratzler shear force value of 43.87 ± 2.57 N, compared to the group that had pH values above 6 which had a mean shear force value of 38.58 ± 3.31 N. This meant that the ostrich meat that had pH_u values above 6 was significantly more tender ($P = 0.009$) than that of the group that had normal pH_u values ($pH < 6$). It is noteworthy that there were only two animals that exhibited a pH_u higher than 6.3. These two animals had pH_u values of 6.34 and 6.32 and also had the lowest (22.64) and 3rd

lowest (27.72 N) shear force values respectively, thus strengthening the concept that when pH_u increases above 6, there is an increase in tenderness of the meat.

Colour

Meat colour is one of the most important criteria when consumers select meat (Warriss, 2000). Consumers tend to select normal-coloured meat and mostly discriminate against meat that is either too pale (PSE) or too dark (DFD) (Ngapo *et al*., 2004). Ostrich meat is perceived as a meat with a dark red colour (Sales & Oliver-Lyons, 1996). There are a number of factors that may contribute to this characteristic of ostrich meat. Firstly, the amount of myoglobin present in the muscle can influence the colour of the flesh, and the high pigment content of ostrich muscle is a characteristic of the ratite species (Sales, 1996). Secondly, the dark colour of ostrich meat is also correlated to the low levels of intramuscular fat (Girolami *et al*., 2003). In addition to the above factors, meat with a high pH_u leads to meat that has a dark colour (Lawrie, 1998).

Table 4.7 indicates all the mean colour ordinates for each of the three treatments. The only differences were between L* (lightness), b* (blue-yellow range) and the hue angle colour ordinates. The control group had a significantly lower mean L* value than the two groups that travelled 60 and 600 km respectively, whilst the latter two groups' L* values did not differ. The lightness (L*) values, in the current study, are somewhat contradictory to the findings of Hoffman *et al*. (2007) who observed that lower L* value were associated with a higher pH_u. The animals, in the current study, that were not transported had the lowest L* value irrespective of the fact that they also had the lowest pH_u values (5.774 ± 0.053) of all three groups. A low b* value is an indication of a product that is less yellow. The control group had a significantly lower mean b* value than the two groups that travelled 60 and 600 km, whilst the latter two groups' b* values didn't differ. The control group exhibited lower hue angle values than the two groups that travelled 60 and 600 km. The two groups that travelled had similar values for all the colour ordinates. This indicates that the two groups of ostriches that travelled produced meat that was lighter and more yellow, although there was no difference in colour intensity (chroma) or redness (a*) in the three groups of ostriches. Fasone *et al*. (2005) only found a significant difference in the L* value of a control (minimum stress) and a stressed group of ostriches. They found that the stressed group of ostriches (34.3 ± 1.9) had a lower ($P < 0.001$) L* mean value than the control group of ostriches (38.1 ± 2.21) - findings contradictory to the findings in the current study.

Table 4.7

Means of the L*, a*, b*, hue-angle (H_{ab}) and chroma values (C*) of ostriches *M. gastrocnemius* for each of the three groups that travelled different distances.

Colour ordinates	Transport Groups (Means ± s.e.)			Comparison of Transport Groups P < |t|		
	Control Group (0 km)	Treatment C (60 km)	Treatment B (600 km)	0 km vs 60 km	0 km vs 600 km	60 km vs 600 km
L*	30.56 ± 0.935	33.57 ± 0.935	35.13 ± 0.935	0.034	0.002	0.252
a*	13.69 ± 0.574	12.04 ± 0.574	12.34 ± 0.574	0.056	0.113	0.717
b*	9.90 ± 0.635	11.84 ± 0.635	12.47 ± 0.635	0.043	0.010	0.494
C*	17.03 ± 0.734	17.07 ± 0.734	17.63 ± 0.734	0.9684	0.5699	0.597
H_{ab}	35.92 ± 1.505	43.87 ± 1.505	45.14 ± 1.505	0.001	0.000	0.556

There was also a positive correlation between the Hue angle and the ultimate pH (P = 0.008; r = 0.528) (Table 4.8), indicating that an increase in pH_u would result in an increase in the hue angles. This is not unusual, since a high ultimate pH would result in very dark meat (DFD) with high hue angle values. If meat has a high ultimate pH, it will lead to a higher iso-electric point of the meat proteins. The proteins will consequently bind more water in the muscle, leading to the fibres being more tightly packed. This will ultimately lead to darker meat as the surface of the more tightly packed meat fibres does not scatter light to the same extent as meat with less tightly packed fibres (normal ultimate pH) (Abril *et al*., 2001).

Table 4.8

Pearson linear correlation coefficients (r) between pH_u and the physical attributes of the three groups of ostriches that travelled different distances prior to slaughter.

Characteristic	r	P < |t|
Cooking loss	0.087	0.688
Drip loss	0.366	0.079
Shear force (kg/1.27 cm diameter)	- 0.293	0.165
L*	0.206	0.335
a*	-0.389	0.060
b*	0.309	0.142
Chroma	-0.043	0.843
Hue	0.528	0.008

CONCLUSIONS

The results in the current study indicate that the birds that travelled furthest had the greatest loss in live weight from the farm to the actual point of slaughter, confirming the belief of farmers that ostriches that travel greater distances to the abattoir have greater losses in live weight. The weight of the digestive systems does not give a clear indication where the major losses occurred during transport and only suggests that birds that did not travel and were kept off feed for a shorter period had greater digestive tract weights. Because of the fact that there were no differences in the digestive tract weights at slaughter of the birds that travelled 60 and 600 km, the weight loss may have occurred in muscles of the birds that travelled 600 km.

The results of this study indicate that ostriches that travelled for longer distances (600 km) produced meat with a higher mean pH_u (measured at 24 hours *post-mortem*) compared to birds that travelled less (60 km) or didn't travel (0 km) prior to slaughter. The extent of pH fall in the 24 hours *post-mortem* was greater in the birds of treatment C that travelled only 60 km, indicating that these birds either had the ability to restore their glycogen reserves after transport or that these birds didn't use their glycogen stores during transport, ultimately leading to meat with a lower ultimate pH. There were significant differences in drip loss between the three treatments. These differences in drip loss are expected. The birds that didn't travel had the least amount drip loss formation due to the fact that their muscles weren't exposed to the physical exertion of transport. Between the two groups of birds that were transported, the group that travelled the furthest had the least amount of exudate formation and this finding can be attributed to the greater pH_u of this group that leads to an increased WHC. The differences in colour between the three groups indicated that the two groups of ostriches that travelled produced meat that was lighter and more yellow compared to the group that didn't travel. This finding doesn't correlate to the mean pH_u values of each group. Because the pH_u of

the two groups was higher than the pH_u of the group that didn't travel, we would expect that the birds in the treatment that didn't travel would yield lighter meat. However, this was not the case and the birds that didn't travel had the lowest L* (lightness), b* (blue-yellow range) and the hue angle colour ordinates. The fact that the $pH_{u,}$ of the birds that travelled the furthest is significantly higher than the other two treatments is an indication that ostrich transportation over long distances adversely affects meat quality in ostriches.

Further research is required to see whether the latter group of birds would be able to restore their glycogen reserves if their lairage period was extended. Further research is also required to see whether birds that travelled long distances to abattoirs would be able to restore their plasma CK and AST concentrations to normal levels which would in turn lead to the production of better quality meat.

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Chapter 5

EFFECT OF *ANTE-MORTEM* STRESS ON OSTRICH (*STRUTHIO CAMELUS*) MEAT AND CARCASS QUALITY

ABSTRACT

Ante-mortem stress is known to adversely affect the meat quality of animals. Transportation of animals also causes bruising which could lead to significant economic losses if transport conditions are not optimal. The purpose of this study was to compare quality of meat, amount of bruising and psychological stress response in ostriches that were raised under various conditions and transported under different conditions to the abattoir. Two hundred ostriches were transported and slaughtered, from three different groups. The groups were divided on the basis of farming system they were raised in namely feedlot, semi-intensive and free range. There was no significant difference in serum corticosterone (ng/ml) as a measure of stress, between treatments. Since the rate of temperature decline of carcasses varied between animals on the slaughter line and chilling room, all pH measurements were adjusted to 4°C. Significant differences were found in drip loss, cooking loss and shear force between treatments. Significant correlations were found between drip loss ($r = -0.456$) and pH_u. A significant difference was found in the percentage carcass weight cut off from the feedlot (0.19 ± 0.032 %) and free range (0.07 ± 0.038 %) treatments due to bruising. The results of this study indicate that stocking density and road conditions could significantly impact on meat quality and bruising of ostriches during transport. These factors, mentioned above, could well have a greater effect on meat quality than factors such as the conditions birds were raised in.

INTRODUCTION

Since ostrich farming started in South Africa in the 1880s (Smit, 1963; Lambrechts & Kruger, 2006), this species has increasingly gained prominence as a livestock animal due to its potential to produce healthy red meat with a low fat content (Cooper & Horbanczuk, 2002). The healthiness of food is of great importance where it regards the preference of consumers (Girolami *et al*., 2003). Ostrich products are currently becoming increasingly popular for export from South Africa to the European Union, with export income amounting up to R1.2 billion (Lambrechts & Kruger, 2006). According to Hoffman *et al*. (2007) information is still required on means to improve the quality of ostrich meat. Pre-slaughter stress is known to have a significant impact on the quality of meat (Owens & Sams, 2000), yet very little research has been conducted on the effects of *ante-mortem* stress such as transport and lairage on the meat quality of this bird.

Three different farming systems (intensive, semi-intensive and extensive) are currently used for ostrich farming in South Africa (Lambrechts & Kruger, 2006; Hoffman & Lambrechts, 2010). Birds in extensive systems are either born in natural veldt conditions where the eggs are incubated by the ostrich itself and raised by their own parents or, the eggs are hatched in an incubator but the birds are moved to an extensive system as soon as possible where they fend for themselves.. The problem with an extensive system is that

these birds are not used to human handling, making them extremely difficult to handle during loading and transportation, which ultimately leads to excessive stress (Lambrechts & Kruger, 2006). Animals subjected to regular intervention by humans become tamer (Neindre *et al*., 1996). These animals are handled easier with less impact on animal welfare. The stress encountered during the handling of ostriches may lead to skin damages and decreases the meat quality of the birds; both these responses can lead to significant economic losses to producers (Hoffman & Lambrechts, 2010). Slaughter ostriches have also been reared in commercial feedlots from the early 1980s. In feedlots, animals become accustomed to workers and handlers because of every day interaction between the handlers and the specific animals, especially during feeding and inspection for diseases and parasites (Hoffman & Lambrechts, 2010). The feedlot ostriches will thus most likely be less affected by stress during periods such as loading and transportation. Wotten and Hewitt (1999) proposed that ostriches should be trained and handled frequently by humans from an early age in order for the birds to become accustomed to their handlers and thereby reduce stress and its adverse effects.

With the exception of the work of Van Schalkwyk *et al*. (2005) and Fasone *et al*. (2005), relatively little research has been conducted on *ante-mortem* stress and its effect on ostrich meat quality (Hoffman & Lambrechts, 2010).

This study was therefore conducted to determine if the type of farming system could lead to differences in the susceptibility of ostriches to *ante-mortem* stress during transportation and lairage under commercial practices.

MATERIALS AND METHODS

Animals

Two hundred ostriches made up three different experimental groups in this trial and were produced from three commercial farms in the Southern Cape. All three groups were slaughtered on the same day at the Mosstrich abattoir in Mossel Bay as part of their normal commercial activities. One group travelled 104 km to the abattoir and was from the farm Stolzvlakte near Oudtshoorn where they were raised in a feedlot (intensive system; feedlot system). The other two groups travelled from Calitzdorp (144 km) (Goedeverwagting) and Heidelberg (148 km) (Diepvlei) and were raised in semi-intensive and free range systems respectively. The trial was conducted from the 21st to 23rd of October 2008. The design of this trial was a completely randomised design with three treatments (different farming systems). Ethical approval for the study was obtained from the Stellenbosch University Sub-committee B Animal Research Ethics committee (reference number, 2009B03003).

Transport and Slaughter

The three groups of ostriches were all slaughtered at Mosstrich abattoir in Mossel Bay on the same day after arriving at the abattoir the previous day. The dimensions of all three trucks used in the trial were determined, as well as the floor type of each truck. From the dimensions on the truck and the amount of birds in each

compartment, the stocking density was calculated for each of the three trucks (Table 5.1). During transport the weather was documented by the truck driver. When the birds arrived at the abattoir, the research team documented the off-loading of the animals along with any incidences of injury.

The first batch of ostriches arrived at the abattoir at 09h00 from Oudtshoorn (feedlot treatment) after having travelled 104 km, and was then placed in lairage pens with access only to water. Due to unforeseen reasons, off-loading of the birds couldn't be supervised. The behaviour of the birds in lairage was monitored from 09h15 until 22h30 that evening.

The second group of ostriches arrived at the abattoir at 11h45 that same morning from Calitzdorp (semi-intensive treatment) after having travelled 148 km. These ostriches were raised on grazing (lucerne) and also received a commercial feedlot diet as supplementation (semi-intensive treatment). There were 100 ostriches in this group, however, 70 ostriches from this group were slaughtered within an hour after arriving at the abattoir. This was done without consent of the management and research team and only 30 ostriches from this group remained that were used in the current study. After the 30 birds were placed in lairage pens the research team also monitored the birds until 22h30 that evening.

The third group of ostriches arrived at the abattoir at about 13h05 from Heidelberg (free range treatment). These ostriches were raised on lucerne (alfalfa) on a free range farming system and were transported 144 km to Mosstrich. There were 70 ostriches in this group and they were immediately placed in lairage pens adjacent to the birds of the semi-intensive treatment where they were monitored until 22h30 that evening.

Table 5.1

A summary of each of the truck descriptions, road conditions, floor space per bird and additional information regarding the transport and off-loading of the birds in the current study.

Treatment Group	Distance Travelled	Number of Birds	Truck Description	Floor Type	Average Floor Space/Bird	Assisting Stockmen	Comments
Feedlot	104 km	100	International 98001 Pro sleeper 2 Wagons	Rubber	0.437 m^2	5	One bird placed under the truck in a special compartment that struggled to stand due to a broken leg during transit (bird removed from experiment and slaughtered immediately according to abattoir procedures). Birds were able to look over the railings of the truck which led to bruising on the neck area. Birds travelled via a mountain pass. Birds had chafing marks on their legs and knees. Three ostriches were injured on the lower part of their necks. The feathers of these birds were plucked prior to transport and this led to some of the birds still having blood on their wing tips and tail.
Semi-intensive	148 km	100	Hino 15-258 2 Wagons	Rubber	0.39 m^2	6	Birds were able to look over the railings of the truck which led to bruising on the neck area. Birds travelled via a mountain pass. Ostriches looked harassed/confused and turned around in the chute. This group urinated and defecated the most of the three groups. One bird's wing was injured. Another bird had a chafing mark on the back of a leg. Two birds had light foot injuries and one bird had a light knee injury.
Free range	144 km	70	Mercedes Antego 1528 2 Wagons	Steel Grids	0.507 m^2	3	Birds were able to look over the railings of the truck which led to bruising on the neck area. Birds travelled via a relatively straight road. One bird fell over and damaged its foot during transit. Struggled to unload the ostriches. Stockmen needed to push birds one by one off the truck onto loading ramp which injured some of the birds as they slipped trying to escape.

The following morning the birds of the feedlot treatment were slaughtered from 07h00 onwards, followed by the birds of the semi-intensive and free range treatments until all 200 ostriches were slaughtered. The birds were slaughtered according to standard ostrich abattoir practices. Bruises were cut off during primary inspection (warm carcass). All the carcasses were individually tagged so that individual carcasses could be distinguished from each other in the abattoir. The following data was obtained from each of the 200 ostriches slaughtered:

Temperature and pH readings were taken whilst all the carcasses were still on the slaughter line 1 hour *post-mortem*. All readings were taken in the right *M. gastrocnemius* (big drum muscle).

Temperature and pH readings were again taken, directly after placing the carcasses in the chiller (about 2 hours *post-mortem*).

The weight of bruised meat trimmed off the carcass was weighed for each carcass.

The following day, the following data was obtained from each of the 200 ostriches slaughtered:

The temperature and pH of the *M. gastrocnemius*, 24 hours *post-mortem* in the chiller.

The cold carcass weight.

Drumstick weight and the percentage (2 drumsticks) thereof relative to the cold carcass weight were calculated for each bird.

The percentage of cold carcass weight that was trimmed off due to bruises was calculated.

Temperature and pH readings were taken with a Testo 205 meter (Testo AG, Germany). The pH meter was calibrated after every ten readings.

Blood samples

One blood sample was collected in a clean gel centrifuge tube, from each ostrich, in a sub-group of 30 birds (10 birds randomly selected from each of the three groups in the trial) within a few seconds after stunning and exsanguination. These samples were marked and kept on ice until reaching the lab. At the lab, the blood samples were analysed by a rodent EIA kit (AC-14F1, Octeia, Immunodiagnostics Systems) to determine the corticosterone concentration of each of the samples.

Physical analysis

The same 30 randomly selected ostriches (10 from each group) used in the blood sample collection described above were also used in the meat analysis trial. After 24 hours *post-mortem*, one large meat sample (≈200 g) from the *M. gastrocnemius* (right-hand side of each carcass) was removed. The following meat analyses were performed on the 30 birds as described by Honikel (1998):

Drip loss percentage

Cooking loss percentage

Shear force values

Drip loss was determined by hanging individually-weighed samples (ranging between 50 g and 100 g) in inflated polythene bags (care was taken that samples didn't touch the sides of the inflated bags) for 24 hours at ± 4°C in a chiller room. After 24 hours, the samples were removed and weighed, and drip loss was calculated as the percentage of weight lost from the sample during the 24 hours the sample spent in the polythene bag. The cooking loss was determined by weighing a second muscle sample (ranging between 50g and 100g) before placing the sample in a polythene bag in a water bath at ± 80°C for 50 minutes. The samples were then removed from the water bath, the water drained from the bags and the samples (still in the bags) cooled under running water to ± 20°C. After the samples were cooled they were removed from the bags and patted dry with absorbent paper and weighed. The cooking loss was then calculated as the percentage of weight lost by each sample during the cooking period.

The cooled meat samples (± 20°C) used in the above-mentioned cooking-loss procedure was then used to determine tenderness using a Warner-Bratzler device, with a load of 2.000 kN, attached to test model 4444 Instron Testing Instrument (Apollo Scientific cc, South Africa). Five cylindrical core samples of 1.27cm diameter each were cut parallel to the grain from each cooked piece of muscle (each muscle representing a different bird) at random locations on the cooked steak. Maximum Warner-Bratzler shear force values were recorded by cutting the cylindrical core of cooked muscle, perpendicular to the longitudinal orientation of the muscle fibres at a crosshead speed of 200 mm/min. An average shear force value (N) was then calculated for each bird. Care was taken to avoid cylindrical core samples that contained visible connective tissue that could influence shear force results.

Statistical analysis

Data was analysed using Statistica v.8. Analyses included ANOVA with the Fisher LSD (least square difference) test as the post hoc test. The ages of the birds were between 8 and 10 months, whilst the gender of the birds was neglected, resulting in the only main effect considered, when analyzing the data, being the treatment effect. The differences between the groups of birds (i.e. treatments) were tested by means of the null hypothesis (H_o), with H_o: $\mu_1 = \mu_2 = \mu_3$ and the alternate hypothesis (H_a) being H_a: $\mu_1 \neq \mu_2 \neq \mu_3$. Data are presented as means and standard errors. A p-value of < 0.05 was accepted as statistically significant**.** A Pearson correlation coefficient was also calculated for the pH_u vs. drip loss, cooking loss and shear force. Proc GLM was also performed on the pH data. In the case of the pH values, all the pH values of all the birds were also divided into categorical data according to the pH value of the birds. The pH categories were pH > 6.2, 6 - 6.2, 5.8 - 5.99 and < 5.8. The percentage of birds that fell into each category was noted. This was done because individual birds differ in their response to stress and for abattoirs to determine the proportion of birds that has high (extreme) pHs that could result in DFD meat.

RESULTS AND DISCUSSION

Bruising of Carcass

The on and off-loading and transportation of animals are the major contributing factors to bruises on carcasses (Grandin, 1990). The bipedal nature of ostriches and the fact that these birds have a high centre of gravity contributes to ostriches struggling to remain upright and balanced during transit (Wotten & Hewitt, 1999). Hallam (1992) (as cited by Wotten & Hewitt (1999)) reported that ostriches bruise easily and this, coupled with the fact that ostriches are more likely to lose balance during transportation (compared to cattle for instance), make these animals more susceptible to bruising during handling and transportation. Other injuries that ostriches suffer during handling and transportation are commonly dislocated and broken hips, broken legs and wings, and internal haemorrhage. In the current study the most bruises (different grades) occurred on the necks and thighs of the ostriches (Table 5.2). Loading and off-loading facilities have been improved during the recent past to decrease bruising of carcasses. Hoffman *et al*. (2010) reported on the distribution of bruises on ostrich carcasses in a commercial abattoir. They reported that more than 50 % of the bruises were on the necks, whilst about 37 % of bruises were on the front of the thighs. The findings of Hoffman *et al*. (2010) correspond to the results in the current study regarding the most prevalent areas on ostrich carcasses where bruising occurred. The heights of all three trucks' sides were such that the heads of the birds protruded, which resulted in them being able to see outside and it is postulate that this may be the reason for such a high bruising incidence on the neck area (Table 5.2). This aspect warrants further research. Transport and handling can also lead to severely traumatised birds that can result in increased mortalities (Wotten & Hewitt, 1999). Mortalities, coupled with the fact that bruised meat is discarded and ostrich skins that are damaged (such as bruises and kicking marks) is downgraded at the ostrich tannery, all lead to major economical losses to the ostrich industry (Wotten & Sparrey, 2002; Hoffman *et al*., 2010).

Table 5.2

The relative percentages of subjective bruise scoring at various locations on the 200 ostriches in each of the three groups in the current study.

Bruise Information	Treatment Groups (%)			Grand Total (%) (n=200)
	Feedlot (n=100)	Semi-intensive (n=30)	Free range (n=70)	
Birds not bruised	37.0	43.3	48.5	42.0
Bruising on thighs (any degree)	26.0	10.0	10.1	18.0
Bruising on necks (any degree)	19.0	36.7	30.0	25.5
Bruising on thighs and necks (any degree)	18.0	6.6	8.5	13.0
Bruising on necks and feet (any degree)	0.0	3.4	0.0	0.5
Bruising on chest (any degree)	0.0	0.0	1.4	0.5
Bruising on chest and neck (any degree)	0.0	0.0	1.4	0.5
Grand Total (%)	100.0	100.0	100.0	100.0

Bruised meat is wasted because it is “aesthetically” unacceptable to consumers and spoils more rapidly due to the fact that the bloody meat is an ideal growth medium for bacteria during storage (Chambers *et al*., 2004). In South African export abattoirs, primary meat inspection on hot carcasses (1 hour after slaughter) is conducted by a qualified meat inspector of the Department of Agriculture. During this inspection, the inspector trims away bruises from the carcass (Hoffman & Lambrechts, 2010). However, Hoffman *et al*. (2010) reported that cold trimming led to a decrease in meat losses compared to the common practice of hot carcass trimming. In the same study they also observed that the microbial load on the meat decreased when the bruise was trimmed from the cold carcass in comparison to bruise trimming on the warm carcasses. McEwen and Barbut (1992) also found a positive correlation between transport time and the incidence of carcass lesions.

Although there were significant differences in cold carcass weight between each of the three groups in the current study (Table 5.3), there is insufficient evidence to suggest that the different systems the birds were raised in accounted for the differences in cold carcass weights. The cold carcass weights in the current study were lower than the average cold carcass weights of the groups slaughtered at the Swartland abattoir (45.03 ± 1.311 kg; Chapter 4). Unlike in Chapter 4, the dressing percentage of each carcass in the current trial could not be calculated due to the fact that the live weights of the 200 ostriches weren’t recorded. The differences seen in cold carcass weights between the current trial and Chapter 4 are most probably due to differences in the live weight of the ostriches in the two different trials.

The amount and percentage of bruises cut off from each of the carcasses during processing were also determined (Table 5.3). On average, the feedlot ostriches lost the most weight (75.92 ± 12.690 g) due to bruised meat that was cut off in the abattoir during processing. The ostriches that were raised on the free range system had the least amount of meat cut off from each carcass (27.23 ± 15.167 g). These 2 groups mentioned above were the only groups that differed (P = 0.015) in the amount of bruises cut off during processing. Hoffman *et al*. (2010) reported average meat losses of more than 250 g when bruises were trimmed from warm carcasses whilst cold trimming led to decreases in the average amount of meat lost (only 130 g) on ostrich carcasses. The feedlot birds also had the highest percentage meat cut off on average from each carcass, and this percentage was significantly higher (P = 0.013) than the percentage meat cut of from the free range ostriches.

It is interesting to note that the group of ostriches that travelled the shortest distance to the abattoir and were supposedly more used to handling by humans (feedlot), had the largest losses in meat due to true bruising. Bruises commonly occur when the stocking density of animals is incorrect or if animals slip and fall and get trampled on. The minimum required floor space per bird when being transported is 0.5 m^2 (Hoffman & Lambrechts, 2010). Birds also should not have too much floor space per bird as this will lead to birds falling because of their unstable bi-pedal nature. In the current study, all three groups had different stocking densities on each of the respective trucks. The feedlot birds (most bruises) had an average floor space of 0.437 m^2/bird whilst the free range birds (least bruises) had an average floor space of 0.507 m^2/bird. The semi-intensive group had the least amount of floor space (0.39 m^2) per bird (Table 5.1). The free range birds was the only group of birds that had the floor space specifications close to that proposed by Hoffman and Lambrechts (2010). This may indicate why this group of birds had the least amount of bruises and why the birds of the feedlot and semi-intensive treatments, which did not have a minimum floor space of 0.5 m^2 had more bruises. However, average floor space per bird is not the only factor that can influence an animal's susceptibility to bruises. Factors such as road conditions, floor type, driver's ability and calmness of the birds and stockmen can also affect the stability of animals whilst being transported. None the less, it is fair to say that producers should transport ostriches at minimum floor space of 0.5 m^2.

Typically, metal gridding or rubber matting is used as the floor types in ostrich transport trucks (Hoffman & Lambrechts, 2010). Rubber floors are preferred because, according to the transporters, the metal grids can injure the toes of the birds. When birds do fall it will cause bruising to the body and damage to skin, logically resulting in a loss of income. Sides of the trucks are always solid. In the current study, the feedlot and semi-intensive groups were both transported in trucks that had rubber flooring, whilst the free range ostriches were transported in a truck which had steel grid flooring (Table 5.1). Of the birds transported in the truck with metal gridding, only one bird had a foot injury due to a cut on the right foot. However, four birds that were transported on trucks with rubber matting had leg injuries. It is assumed that these birds all injured their legs/feet whilst in transit as the RSA Code of Conduct does not allow the loading of injured birds (South

African Ostrich Business Chamber, 2001). The type of flooring in ostrich transportation trucks warrants further research.

Three different truck drivers were used to transport the different groups in the current trial. The ability of the truck drivers couldn't be assessed during the trial, but it is interesting to know that both the semi-intensive and feedlot groups, from Calitzdorp and Oudtshoorn respectively had to travel over a mountain pass, the Robinson Pass that connects Oudtshoorn (Route 62) and Mossel Bay. The Robinson Pass has a large number of sharp bends and corners and this could have been one of the main reasons why the two groups of ostriches that travelled over this pass had the greatest amount of bruises. The free range birds which travelled from Heidelberg were transported via the N2, a broad road that has no passes and is very straight in comparison to the Robinson pass.

Another factor that also has the ability to influence the postural stability of ostriches whilst in transit is the number of stockmen/helpers that assist the birds being transported on the truck when the birds fall (Hoffman & Lambrechts, 2010). The free range group only had three stockmen on the truck during transit to assist 70 birds, while the feedlot and semi-intensive groups had five and six stockmen on each truck respectively for 100 ostriches (Table 5.1). The fact that the groups that had the most bruises also had the most workers on the truck during transport may indicate that the workers could have been a cause of stress to the birds and led to the birds trying to escape during transport, thereby injuring themselves and being bruised.

Table 5.3

Mean weights of the cold carcasses, drumsticks and grams cut off during inspection along with the mean % drumsticks (of carcass) and the % grams cut off from 200 ostriches slaughtered at Mosstrich.

Weights	Treatments (Means ± s.e.)			Treatments Compared P < |t|		
	Feedlot (104 km)	Semi-intensive (148 km)	Free range (144 km)	Feedlot vs Semi-intensive	Feedlot vs Free range	Semi-intensive vs Free range
Cold carcass weight (kg)	41.08 ± 0.240	40.93 ± 0.439	39.49 ± 0.287	0.764	0.000	0.007
Drumstick weight (kg)	15.93 ± 0.991	16.07 ± 1.809	15.44 ± 1.193	0.050	0.754	0.050
Drumstick % of carcass	77.54 ± 4.624	78.53 ± 8.441	78.55 ± 5.566	0.050	0.889	0.050
Grams of bruises cut off	75.92 ± 12.690	47.1 ± 23.168	27.23 ± 15.167	0.277	0.015	0.474
% of carcass weight cut off	0.19 ± 0.032	0.12 ± 0.058	0.07 ± 0.038	0.265	0.013	0.473

Corticosterone

The release of glucocorticoids, like corticosterone, is increasingly being used as a way to measure stress in animals (Romero, 2004). When an animal is subjected to stressful or adverse stimuli, an integrated neuroendocrine response takes place that involves the activation of the hypothalamic-pituitary-adrenal (HPA) axis. When the HPA axis is activated, it stimulates the anterior pituitary gland to produce adrenocorticotrophin (ACTH). The ACTH, in turn, is proliferated in the blood and stimulates the synthesis of hormones such as corticosterone (Siegel, 1980). A previous study involving ostriches, which used corticosterone as a measure of stress, was conducted by Mitchell *et al*. (1996). Gross & Siegel (1983) postulated that corticosterone was a better indicator of short-term changes in the environment of an animal, than long term changes.

A summary of the corticosterone results obtained is depicted in Table 4. No blood samples were taken at baseline (i.e. before the birds were transported from the farm to the abattoir), and hence corticosterone changes over the *ante-mortem* period could not be assessed. The magnitude response of corticosterone could therefore also not be calculated. There were no significant differences between the three groups' corticosterone values after they were slaughtered. The corticosterone concentrations in the current study are significantly lower than the baseline corticosterone concentration (16.32 ± 2.82 ng/ml) of the ostriches in the control group of ostriches in Chapter 3. Mitchell *et al*. (1996) and Leche *et al.* (2009) also reported high baseline concentrations in ostrich blood (4.9 ± 2.9 ng/ml) and Greater Rheas (3.98 ± 1.04 ng/ml) respectively. The corticosterone concentration (12.43 ± 2.93 ng/ml), after stunning and exsanguination, of the control group in Chapter 3 is still significantly higher than all three groups' concentrations in the current study. This is a somewhat unexpected finding, since the three groups discussed in this chapter were all transported over a 100 kms to the abattoir and then spent one night in lairage, which is novel to these birds. It was expected that the birds in the current trial would exhibit corticosterone concentrations greater than that of the control group in Chapter 3 due to the fact that the control group in Chapter 3 did not encounter periods of stress such as transport and lairage.

Ratites, like humans, are non-nocturnal species and have a diurnal rhythm of corticosterone (cortisol in the case of humans) over a 24-hour period (Van Cauter, 1989; Leche *et al*., 2009). This diurnal rhythm can influence the corticosterone concentration during different periods of the day. However, the control groups baseline and post-slaughter blood samples (Chapter 3) were taken in the morning between 08h00 and 09h00, as were the blood samples used in the current study to calculate corticosterone concentrations. It is thus highly unlikely that the diurnal rhythm of corticosterone is the cause of the differences between corticosterone concentrations of the birds in Chapter 3 to the birds in the current trial.

The fact that there were no differences between the mean corticosterone concentrations of the three groups of ostriches in the current trial, may be an indication that the amount of acute stress suffered by each treatment group during the *ante-mortem* period was probably of the same magnitude. This

may be an indication that each group of birds in the current trial was equally susceptible to *ante-mortem* stress, regardless of the farming system they were raised under. If one group in the current study encountered more stress during the *ante-mortem* period, compared to the other groups, this stress was not manifested in increased levels of corticosterone or alternatively, the birds in the current trial were able to recover from stress during lairage. This aspect (recuperation period) requires further investigation.

Table 5.4
Mean Corticosterone concentration measured in the blood samples of the three groups of ostriches after stunning and exsanguinations.

Treatments	Corticosterone (ng/ml) (Means ± s.e.)	Treatments Compared	P < |t|
Feedlot (104 km)	1.41 ± 0.254	Feedlot vs Free range	0.332
Free range (144 km)	1.05 ± 0.254	Feedlot vs Semi-intensive	0.699
Semi-intensive (148 km)	1.55 ± 0.254	Free range vs Semi-intensive	0.182

pH

Meat quality is influenced to a large extent by the rate of pH decline and ultimate pH (pH_u) in muscles during the *post-mortem* period (Sales & Mellet, 1996). Ultimate pH (pH_u) is often used as an indicator of the occurrence of DFD (dark, firm and dry) meat. Sales and Mellet (1996) classified ostrich meat as an intermediate type meat due to its pH that ranges between normal (pH < 5.8) and extreme DFD (pH > 6.2). They also concluded that ostrich meat had a high ultimate pH regardless of pre-slaughter handling and stress and that the high pH of ostrich meat was a characteristic of this species. In fact, Hoffman, Botha & Britz (2007) indicated that in some instances, ostrich muscle pH decreased and then increased again during the early *post-mortem* period. Excessive stress during the *ante-mortem* period of animals leads to the depletion of glycogen reserves that in turn leads to less lactic acid production in the muscles during the *post-mortem* period (Balog & Almeida Paz, 2007). The lower production of lactic acid results in higher meat pHs and ultimately DFD meat. Ultimate pH has the ability to affect other meat quality attributes such as tenderness, water-holding capacity and colour (discussed later). A high pH_u (pH > 6) also has the ability to decrease shelf life of meat products due to the enhanced growth of psychrophilic bacteria at high pH (Sales & Oliver-Lyons, 1996). These bacteria promote microbial growth that eventually leads to foul odours (Balog & Almeida Paz, 2007). Although relatively little information exists on the effect of *ante-mortem* stress on ostrich meat quality, a few authors have conducted research on this topic (Fasone *et al*., 2005; Van Schalkwyk *et al*., 2005). Berge *et al*. (1997) also observed a relationship between elevated pH_u and physical stress, due to transport in emus.

When the pH_u of all 200 ostriches of the three groups in the current study was analysed, a difference was found (P = 0.009) only between the feedlot and semi-intensive groups (Table 5.5). However, the pH_u values of the ostriches in this study correspond to the pH_us in Chapter 4. The control group of ostriches (Chapter 4) that was exposed to minimum *ante-mortem* stress had a slightly lower mean pH_u value than all the groups in the current trial. According to Sales and Mellet (1996), a high pH_u is a common phenomenon in ostrich muscles regardless of *ante-mortem* conditions and handling, and said authors proposed that pH_u values between 5.8 and 6.2 were normal for this species. Fasone *et al*. (2005) reported pH_u values of 5.94 ± 0.08 and 6.96 ± 0.17 respectively for a control (not stressed) group and a stressed group of ostriches respectively. The significantly greater pH_u observed by Fasone *et al*. (2005) in the stressed treatment group can probably be related to higher levels of stress suffered by these ostriches during transport and stunning due to factors such as broken limbs and insufficient stunning.

Table 5.5

Mean pH_u values measured at 24 h *post-mortem* for the three groups of ostriches (N = 200) that were raised under different management systems.

Treatments	pH_u (Means ± s.e.)	Treatments Compared	P < |t|
Feedlot (104 km)	5.95 ± 0.018	Feedlot vs Free range	0.059
Free range (144 km)	6.00 ± 0.021	Feedlot vs Semi-intensive	0.009
Semi-intensive (148 km)	6.04 ± 0.033	Free range vs Semi-intensive	0.246

It cannot be determined with certainty that the system under which each of the groups was raised had any effect on the birds' susceptibility to stress in the *ante-mortem* period, which could have influenced glycogen depletion and ultimately affected the pH_u of the meat. Other factors such as road conditions, driver ability, stocking density and floor type could also have influenced glycogen depletion during travel. Interestingly, the feedlot birds had the lowest pH_u and the semi-intensive birds had the highest pH_u of all three the groups, although both these groups travelled the same route (Robinson pass, as mentioned earlier). It seems as if the birds in the semi-intensive group stressed more under the same road conditions due to their inherent wildness compared to the more tame nature of the feedlot birds, and consequently suffered greater depletion of glycogen reserves. The pH_u values of all 200 ostriches were not normally distributed, but when both non-parametric and parametric tests were performed on the data, it yielded the same results i.e. that the pH_u of the three treatments in the current study did differ. Proc GLM was therefore performed on the data. Table 5.6 depicts the pH categories and the percentage of pH values that fall into each of these categories of all three groups of ostriches at each of the three time points. From Table 5.6 it is evident that the data of the pH_us are not evenly distributed. In each group of birds, roughly 50 % had pH_u values in the 5.8 - 5.99 category, whilst the semi-intensive group had the greatest percentage of pH_u values (23.3 %) above the 6.2 pH range.

Table 5.6

Summary of the pH categories of the $pH_{(30\ min)}$, $pH_{(1\ hour)}$ and pH_u of each of the three groups of ostriches (N = 200) that were raised under different management systems prior to being slaughtered.

Time point	pH category	Treatment Group (%)			Grand Total (%) (n=200)
		Feedlot (n=100)	Semi-intensive (n=30)	Free range (n=70)	
Slaughter line (30 min)	pH > 6.2	9.0	6.7	4.3	7.0
	pH 6 - 6.2	15.0	16.7	18.6	16.0
	pH 5.8 - 5.99	59.0	36.7	40.0	50.0
	pH < 5.8	17.0	40.0	37.1	27.0
Chiller (1h)	pH > 6.2	20.2	10.0	2.9	12.1
	pH 6 - 6.2	34.8	23.3	15.7	26.0
	pH 5.8 - 5.99	41.6	63.3	67.1	54.5
	pH < 5.8	3.4	3.3	14.3	7.4
Chiller (24h)	pH > 6.2	11.0	23.3	11.4	13.0
	pH 6 - 6.2	17.0	23.3	38.5	25.5
	pH 5.8 - 5.99	47.0	53.3	47.1	48.0
	pH < 5.8	25.0	0.0	2.9	13.5

Although there were differences in the pH_u values of the three groups of ostriches (of all 200 birds), there were no significant differences between the pH_u of the three different groups in the subgroup of 30 ostriches (Table 5.7).

Table 5.7

Mean pH_u values measured at 24 h *post-mortem* for the three groups of ostriches (N = 30) that were raised under different management systems.

Treatments	pH_u (Means ± s.e.)	Treatments Compared	P < |t|
Feedlot (104 km)	5.90 ± 0.055	Feedlot vs Free range	0.303
Free range (144 km)	5.98 ± 0.055	Feedlot vs Semi-intensive	0.133
Semi-intensive (148 km)	6.02 ± 0.055	Free range vs Semi-intensive	0.621

Water-holding capacity and tenderness

Fresh meat contains about 75 % water and the ability of the meat to retain its water content is termed the water-holding capacity (WHC) (Offer & Trinick, 1983). The moisture content of meat can change, depending on how the product is handled during processing, or due to biochemical factors such as pH (Huff-Lonergan & Lonergan, 2005). Large amounts of purge lead to unacceptable products, whilst purge losses also result in lower meat weights that have a negative effect on income. Guignot *et al*. (1993) reported that the higher the pH_u of the meat, the less moisture would be released from the muscle. Hamm (1986) observed that the WHC of meat is at a minimum between pH 5.0-5.5 and that at this pH, the iso-electric point of the muscle proteins is reached. Thus, if the pH_u of meat were to increase, it would lead to a decrease in the amount of purge. A high pH_u can be attributed to increased *ante-mortem* stress and the depletion of glycogen reserves during this period (Schaefer *et al*., 1995).

Table 5.8 depicts the mean values of the water-holding capacity and the shear force values for the three groups of ostriches. The feedlot ostriches had a greater drip loss than both the semi-intensive (P = 0.001) and free range birds (P = 0.004), whilst the latter two groups did not differ from each other. The percentage drip loss seen in the current study is in the same range as the drip loss values in Chapter 4. The control group in Chapter 4 had the least amount of drip loss (0.40 ± 0.068 %) whilst treatment C and B had values of 1.36 ± 0.068 % and 0.97 ± 0.068 %, respectively. Van Schalkwyk *et al*. (2005) found no difference in drip loss between birds that spent 24 and 56 hours in lairage, before being slaughtered, after each group travelled 10 km prior to lairage. However, Van Schalkwyk *et al*. (2005) observed slightly greater drip loss in the birds that remained for 24 hours (2.18 ± 0.24 %), than those who did 56 hours (1.88 ± 0.26 %) in lairage. Hoffman *et al*. (2007) found similar drip loss values in the *M. gastrocnemius* of South African Black ostriches when they observed a drip loss percentage of 2.1 ± 0.79 %.

As was the case with the percentage drip loss in the current study, the feedlot birds also exhibited the greatest percentage cooking loss in the *M. gastrocnemius*. The feedlot ostriches had a greater cooking loss than the birds in the semi-intensive (P = 0.030) and free range groups (P = 0.028), whilst the latter two groups didn't differ from each other. The results in the current study also agree with the

results in Chapter 4 on cooking loss. This was especially true for the groups that travelled prior to slaughter in Chapter 4, with the birds that travelled 60 and 600 km having drip loss percentages of 38.06 ± 2.017 % and 36.91 ± 2.017 % respectively. The control group in Chapter 4 had a cooking loss of 32.50 ± 2.017 %. Hoffman *et al*. (2007) also reported similar cooking loss values (38.0 ± 1.29 %) to the current study, whilst Van Schalkwyk *et al*. (2005) reported values of 30.8 ± 1.2 % and 29.1 ± 1.3 % respectively for birds that spent 24 and 56 hours in lairage prior to slaughter. Fasone *et al*. (2005) reported significantly lower cooking loss values for control and stressed ostriches, than the current study when they observed values of 23.46 ± 3.86 % and 19.46 ± 4.97 % respectively for each of the groups.

pH_u has an effect on the water-holding capacity of meat. The differences seen between the three groups' pH_u (of all 200 birds) in the current study (Table 5.5) could well have led to the differences seen in the water-holding capacity (observed as the differences in cooking and drip loss) of the birds. The feedlot birds had the lowest water-holding capacity (highest drip and cooking loss), as well as the lowest pH_u of all the groups. These findings support the study of Guignot *et al*. (1993), who reported that meat with higher pH_us would release less moisture from their muscles. A negative correlation ($r = -0.456$; $P = 0.012$) (Table 5.9) between the percentage drip loss and the pH_u of the 30 ostriches slaughtered and used for meat quality analysis was also observed (Figure 5.1). Due to the fact that the feedlot ostriches were probably more accustomed to handling and contact with humans, it would be expected that these birds would stress less and remain calmer during travel. As transport is regarded as a physically demanding factor that can lead to muscle damage, a shorter distance travelled could decrease said effects. It would thus be expected that the feedlot ostriches would have less damage to their muscles and would therefore exhibit lower drip loss and cooking loss values. However, this was not the case and the feedlot ostriches had significantly higher drip and cooking loss percentages compared to the other two treatments. These findings could possibly be attributed to the fact that the feedlot ostriches had the largest incidence of bruises during transit. This may indicate that this group endured greater physical stress and muscle damage during transport due to the specific conditions of the road and truck during transit.

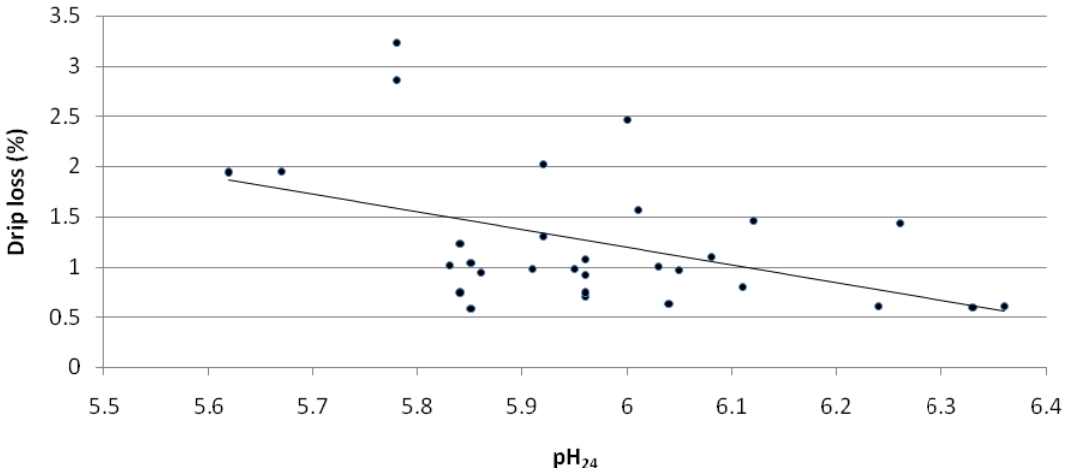

Figure 5.1 Pearson linear correlation (P = 0.012; r = -0.456) between the percentage drip loss and the ultimate pH after 24 hours, *post-mortem*, in all 30 ostriches that travelled to the abattoir and were used in meat quality analysis (independent from treatment).

One of the most important sensory factors affecting consumer preferences in meat, is tenderness (Resurreccion, 2003). There is still controversy over the effect pH_u has on meat tenderness. There seems to be a curvilinear relationship between the ultimate pH and the tenderness of meat that could possibly explain why there is no consensus on this subject (Purchas, 1990; Devine *et al*., 1993). Both these groups of authors reported that tenderness decreased as the pH_u increased from 5.5 to 6.0, whilst meat with pH_u above 6.0 becomes more tender as the pH_u increased to 7.0. Yu and Lee (1986) attributed the increased tenderness of the meat at high pH_us (pH_u > 6), to the greater calpain activity in meat at a neutral pH, *post-mortem*. They concluded that the increased tenderness observed at low pH_us (pH_u < 5.5) be attributed to greater protease activity at an acidic pH_u.

The semi-intensive ostriches had a lower shear strength value (P = 0.028) than the ostriches that were raised on the free range system. There were no other differences in shear force values between the groups. The shear force values from Chapter 4 are slightly lower than that of the current study, with the control group, treatment C and treatment B having mean values of 44.51 ± 3.597, 38.41 ± 3.597 and 42.74 ± 3.597 N respectively. Fasone *et al*. (2005) reported Warner-Bratzler shear force values of 6.13 ± 2.05 and 7.11 ± 1.72 kg F cm^{-2} for a stressed and a control group of ostriches, whereas Van Schalkwyk *et al*. (2005) reported values of 0.078 ± 0.006 and 0.067 ± 0.007 N/1.27 cm^2 respectively for birds that spent 24 and 56 hours in lairage, prior to slaughter. Hoffman *et al*. (2007) reported a shear force value of 63 ± 10.6 N for South African Black ostriches in the *M. gastrocnemius.*

Shear force of meat is influenced by temperature, rate of pH decline, as well as pH_u. The semi-intensive birds had both the lowest shear strength values and the highest pH_u of all three groups in the current study. The differences in pH_u between the three groups could have influenced the shear force values in the current study. These findings agree with the study of Devine *et al*. (1993) who

reported that meat became more tender as the pH_u of the meat increased above 6. However, there was no linear correlation between pH_u and shear strength in the current study (Table 9), and the unique curvilinear relationship of pH_u and tenderness described earlier may be a possible reason for this finding. This was also the case in Chapter 4. When the subgroup of 30 ostriches in the current trial was divided into two groups, namely those with pH_u values below 6 (normal pH) and those with pH_u values greater than 6.0 (DFD pH), the ANOVA revealed that there were no differences in shear force values between the two groups. It is noteworthy that the two animals that exhibited the highest (pH_u = 6.36) and second highest pH_u (pH_u = 6.33) had respectively the 3rd lowest (39.632 N) and lowest average (29.848 N) shear force values of all 30 birds in the current trial, thus strengthening the concept that as pH_u increases towards 7, there is an increase in tenderness of the meat.

Fasone *et al*. (2005) reported that stressed ostriches clearly had lower shear strength values than ostriches that were not stressed; these stressed ostriches had a higher pH_u than the control group and this may well have influenced the tenderness due to the greater calpain activity at pHs nearer to neutral (Yu & Lee. 1986). Although Hoffman and Fisher (2001) found that shear force values increased with age in ostriches (10 months vs 8 years), it is highly unlikely that age made any difference in the shear force values in the current trial due to the fact that all the birds involved were 8-12 months old.

Table 5.8

Mean Drip loss, Cooking loss and Warner-Bratzler shear force values as measured in the *M. gastrocnemius* of the three groups of ostriches that were raised under different management systems.

MeatQuality Parameters	Treatment Groups (Mean ± s.e.)			Treatments Compared P < |t|		
	Feedlot (104 km)	Semi-intensive (148 km)	Free range (144 km)	Feedlot vs Semi-intensive	Feedlot vs Free range	Semi-intensive vs Free range
Drip loss (%)	1.83 ± 0.176	0.88 ± 0.176	1.05 ± 0.176	0.001	0.004	0.502
Cooking loss (%)	40.47 ± 0.694	38.22 ± 0.694	38.19 ± 0.694	0.030	0.028	0.978
Shear force (N)	53.15 ± 3.400	45.78 ± 3.340	56.98 ± 3.340	0.137	0.433	0.028

Table 5.9

Pearson linear correlation coefficients (r) between pH_u and the physical attributes of the 3 groups of ostriches.

Characteristic	r	P < |t|
Cooking loss	-0.323	0.082
Drip loss	-0.456	0.012
Shear force (kg/1.27 cm diameter)	-0.211	0.262

CONCLUSIONS

In the current study the feedlot birds had the largest percentage of meat cut off from each carcass. There are a number of factors such as floor space, road conditions, driver capabilities, type of floor and the calmness of the birds during travel that could possibly affect the birds' susceptibility to bruising during transport. The minimum required floor space per bird when being transported is 0.5 m^2, and this requirement seems to be correct due to the fact that the free range birds were the only ones transported in accordance with these standards and were also the ones that suffered the least amount of bruising. This is not clear cut, the route from Heidelberg was also the road without the Robinson mountain pass and probably led to much 'smoother' transportation of the free range birds. Unfortunately the experimental design was more of a case scenario and allowed for much more variables than the main effects that was studied like road conditions, ostrich density, distance, driver skills, production systems.

The results of the current study indicate that there were no differences between the three groups' corticosterone values after they were slaughtered. This finding probably indicates that the three groups of ostriches in the current trial experienced the same levels of acute stress during the *ante-mortem* period and that the system wherein the ostriches were raised did not lead to any differences in corticosterone concentrations (as a measure of stress). However, the results in the current study were significantly lower than the corticosterone concentrations measured in Chapter 3. The reason for this is unknown. If one group of birds in the current trial did encounter more stress during transport and lairage, the stress experienced was not manifested in increased levels of corticosterone. Alternatively, the birds were able to recover from stress during lairage – more research is required to monitor the duration of lairage on stress recuperation in ostriches.

There were differences between the three groups' pH_us. However, their pH_us were in the same range as the pH_us in Chapter 4. The feedlot birds had the lowest pH_u. The results were inconclusive as it could not

confirm that production system influenced the susceptibility of ostriches to stress and hence glycogen depletion and ultimately the pH_u of the meat.

There were differences in the water-holding capacity (cooking and drip loss) between the groups in the current trial. The feedlot ostriches that were more used to human contact and travelled the shortest distance, had the greatest percentage drip and cooking loss. However, there were no differences between the three groups' pH_u, and therefore pH_u cannot account for the differences in cooking and drip loss in the current trial, although there was a negative correlation between the percentage drip loss and the pH_u. The feedlot ostriches that had the most bruises during transit also had higher drip and cooking loss, indicating that this group most probably suffered the greater muscle damage during transport. A more detailed physiological evaluation of cellular damage may elucidate this phenomenon.

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Chapter 6

GENERAL CONCLUSIONS

CONCLUSIONS

There was no difference between the 60 and 600 km groups, nor within the groups over time in the plasma levels of total protein, globulin and albumin. This is an indication that dehydration and the immobilisation of body reserves through protein catabolism did not occur when the ostriches were transported to the abattoir and deprived of feed and water for a certain period. The WBC count increased over time within each group, whilst the groups at each time point did not differ from each other. The increases seen in the WBC count during the *ante-mortem* period is an indication that there was a degree of cell damage that led to an inflammatory response.

Transport of 600 km caused a significant increase in the H:L ratio from pre-transport to post-transport, whilst no differences in the H:L ratio were seen at any stage in the birds that travelled 60 km. At all stages during the trial, except after transport, there was no difference between the two groups' H:L ratio. The H:L ratio is known to be an accurate assessor of stress and is possibly an indication that the birds that travelled 600 km encountered more stress during transport. An increase in the H:L ratio during periods of stress may possibly affect the immune status of the bird, rendering them more susceptible to diseases.

The birds that travelled 60 km had significantly greater levels of plasma corticosterone at every stage of the trial except at baseline. The highest corticosterone concentration was, however, seen prior to transport when the birds (60 km group) had not even been exposed to transport. On the other hand, the birds of the 600 km group had similar plasma corticosterone levels at each phase during the protocol without any increases in plasma corticosterone levels from baseline values. Elevated plasma corticosterone levels are also an indication that ostriches suffer from acute stress and would suggest that, prior to transport, the birds of the 60 km group probably suffered from an acute stress exposure that was not related to transport. Unfortunately, at this time, a definite conclusion regarding the effect of transport on ostrich plasma corticosterone cannot be drawn.

There were differences in effects that no travel, short travel and lengthy travelling distances had on the extent of *post-mortem* pH decline or meat quality in ostriches. Increased travelling distances led to ostrich meat with increased pH_u and decreased water-holding capacity. Distance travelled also affected the amount of live weight lost by ostriches, with the birds having travelled the furthest to the abattoir exhibiting the greatest live weight loss. This finding confirms the belief of farmers that birds that travel greater distances lose more weight than those that did not travel, regardless of the time they spent off feed.

There were differences in pH_u of ostriches that were raised in different systems and then transported before being slaughtered. As expected, feedlot ostriches had the lowest pH_u *post-mortem,* probably due to the fact

that they were more accustomed to people and stressed less during transport, whilst the semi-intensive ostriches had the greatest pH_u. However, the feedlot ostriches had the lowest water-holding capacity, which could have been attributed to the increased incidence of bruising on the carcasses of the feedlot ostriches compared to the extensive and semi-intensive ostriches. It seems as if the amount of bruising cut off from carcasses during meat inspection is not dependant on the farming system the birds were raised in, but rather due to factors such as stocking density on the truck, road conditions and truck flooring. In the case of the feedlot birds, their stocking density was not appropriate, added to which the road they travelled on consisted out of sharp turns and bends that may have resulted in postural instability and consequently led to bruising.

SUMMARY OF CONTRIBUTIONS

The manner in which we calculate stress in various species is not standard. By using blood haematology to determine stress levels, there must be definite indicators of both physical stress and psychological stress in ostriches. Some haematological metabolites may prove better indicators of physical stress and others better indicators of psychological stress. When the two groups of ostriches were transported for 60 and 600 km respectively, there were different trends in the concentration of the blood metabolites corticosterone and H:L ratio, both of which are indicators of stress in avians. The birds that travelled 600 km had an increased H:L ratio (stress) during the *ante-mortem* period, whilst the ostriches that travelled 60 km hadn't. With regards to corticosterone, the ostriches that travelled 60 km were the only group that exhibited an increased plasma corticosterone concentration (stress). However, the increased corticosterone levels occurred prior to transport and can thus not be related to transport. Another factor could possibly have led to the increased corticosterone levels. Further studies are needed to make definite claims on the type of stress (physical or psychological) leading to increased corticosterone and an increased H:L ratio.

When considering the H:L as an indicator of stress, lengthy transportation of slaughter ostriches caused more *ante-mortem* stress than shorter travelling distances. This was also evident in the meat quality of these ostriches, with the ostriches that travelled further having greater *post-mortem* pH_u.

Transportation as a whole caused increases in WBC counts and the enzymes s-AST and s-CK. This is an indication that the transported ostriches suffered muscle damage during transit that could influence the meat quality of the ostriches

Regardless of feed deprivation period, transport distance affects live weight loss, with the longer periods of road travel resulting in greater live weight losses that ultimately lead to a reduced income to the producer.
Road conditions truck specifications had a definite effect on ostrich meat quality and on the amount of bruising caused during transit.

FUTURE RESEARCH

A sedative may help to decrease the negative effect that acute stressors have on the welfare of ostriches whilst they are being transported to an abattoir or to another farm, and warrants further research.

Transport of ostriches at times when traffic is at a minimum will help to alleviate stress, although it will have no effect on metabolic stress. In other studies, transport of ostriches at night has shown that birds tend to sit down and are more relaxed when they travel in darkness. This requires further research.

Further research is needed to determine where live weight losses take place in ostriches during transport, specifically whether the losses are due to faecal output or due to losses in the muscle. Research is also required to decrease live weight losses by using supplements such as electrolyte solutions that have been tested in cattle.

Greater research is needed on the changes that occur in the haematology of ostriches under stress to determine which metabolite is related to physical stress and which metabolite is related to psychological stress.

More research is needed on the optimal truck conditions ostriches must be transported under to reduce stress susceptibility, bruising and mortalities and improve meat quality *post-mortem*. These factors should include truck floor type, road conditions, driver capabilities and bird density on the truck.

Research is also needed to prove whether farming system affects stress susceptibility and meat quality of birds.